ANALYTICAL METHODS

IN

ORGANIC CHEMISTRY

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BY

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FUNCTIONAL GROUP ANALYSIS BY CHEMICAL METHODS

PART TWO

SOME APPLICATIONS OF SP ECTROSCOPIC METHODS

OF ANALYSIS

FUNCTIONAL GROUP ANALYSIS
BY
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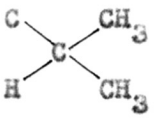
CHAPTER - I

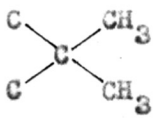
DIFFERENTIAL KUHN-ROTH C-METHYL ESTIMATION

DIFFERENTIAL KUHN-ROTH C-METHYL ESTIMATION

An important item of information sought for the structure determination of organic natural products, especially isoprenoids, is the number and nature of C-methyl groups. C-methyl determination by the Kuhn-Roth chromic acid oxidation method[1] proved of considerable consequence. Later, infrared spectrophotometry provided a superior method, when suitable reference compounds could be used[2-6]. In recent years, this information is most conveniently and effectively provided by PMR (proton magnetic resonance) spectrometry. However, often situations arise when further refinement of the information becomes valuable. Thus, often it is desirable to establish whether the two methyl groups which show up in a PMR spectrum as sharp signals, are present as I, II or III.



I



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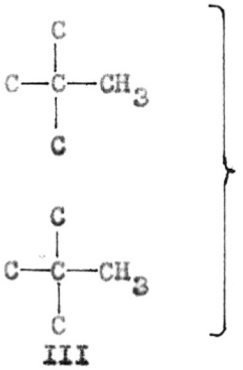
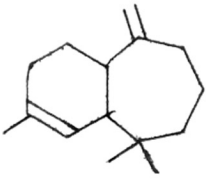


III

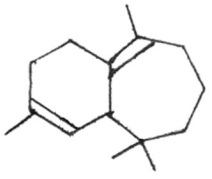
Though PMR spectrometry can be employed to distinguish

between the situation I and II, one will have to make measurements at two different frequencies. On the other hand, a distinction between II and III, in an unknown, cannot be made by the existing methods*.

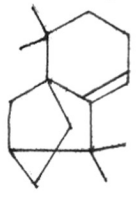
During our work on the structure determination of himachalenes IV, V and isolongifolene VI[8-11],



IV



V



VI

we were faced with similar situations, and we now report a method, based on Kuhn-Roth C-methyl estimation at two different temperatures, which provides the answer in the field of sesquiterpenoids.

KUHN-ROTH C-METHYL ESTIMATION

The Kuhn-Roth method for the determination of the methyl group attached to carbon is based upon its

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*In principle, this distinction is possible by IR spectrometry and this method has been used in certain cases[7]. However, the results are often viciated due to the presence of other methyl groups and due to general variation in the ε value of the methyl group.

oxidation to acetic acid with a chromic acid-sulphuric acid mixture. Under the conditions employed the oxidation of acetic acid by the reaction mixture is relatively slow as compared to the oxidation of the sample to acetic acid. When a compound containing one or more methyl groups is oxidised, a quantity of acetic acid is formed which appears to be dependent on the structure of the compound. It has been observed by Barthel and La Forge[12] that theoretical values are seldom obtained except with certain types of groupings. Although, in general, the side chains of compounds furnish theoretical yield of acetic acid with good precision, other groupings, such as a methyl group attached directly to an alicyclic ring or an aromatic ring and more than one methyl group on the same carbon atom invariably fail to yield quantitative amounts of acetic acid. Typical compounds containing one or more methyl groups attached to carbon atoms having different structural environments showed that the yields varied from 0.29 to 0.85 moles of acetic acid per methyl group.

Unsatisfactory yields of acetic acid from certain compounds led to further investigations in the procedure with respect to the time and temperature and changes in

the apparatus design, reagents etc. Digestion of the compound with the reaction mixture in sealed tubes has been used to avoid losses due to certain volatile compounds formed during oxidation; mechanical agitation of the sealed tube has been used to ensure continuous mixing of a sparingly soluble solid, an immisible liquid or a gaseous organic compound with the chromic acid-sulphuric acid solution[13,14]. Weisenberger has shown the relation of the oxidisability of certain compounds with the time of reaction[15]. The reaction temperature also seems to be rather critical and the use of higher temperature (150°C) has resulted in some destruction of the acetic acid formed[14]. The yields of acetic acid could be improved in certain cases by using more concentrated reagents. Ginger[16] observed that by increasing the proportion of concentrated sulphuric acid in the reagent, the oxidation of the branched chain fatty acids was better and the overall yield of acetic acid per terminal methyl group had improved to about 75-85% of the theoretical value . Jacketed stills designed by Weisenberger[15], Schöniger et al[17] and Tashinian and his coworkers[14] for rapid distillation of the acetic acid saved considerable time for the estimation. Weisenberger passed the vapours of the volatile compounds formed during

oxidation through a hot solution of chromic acid-sulphuric acid mixture for their better conversion to acetic acid[18]. Neutralisation of the excess chromic acid before distillation of the acetic acid using hydrazine or hydrogen peroxide[19] has, later, been shown to be unnecessary[14] and direct distillation of acetic acid is possible without its loss due to oxidation. A comparison of four different methods for the estimation of the methyl group attached to carbon has been studied by Gore and Gupte[20], who have concluded that the digestion of the compound in a sealed tube is most effective.

Karrer and coworkers[21] have developed a method for the estimation of the methyl group linked to carbon with 0.2 to 0.4 mgm substance, which includes treatment of the acetic acid formed with ethylamine solution and its further estimation with the help of paper chromatography.

METHODS AND PROCEDURES: Although attempts have been made to use the Kuhn-Roth C-methyl determination and its modifications for deciding the nature of the methyl group, its use has been limited due to the doubtful inferences drawn from the yield of acetic acid, especially

in the case of compounds containing several methyl groups of different types, and also those containing gem-methyl groups[22,23].

As a result of a detailed study of the determination of C-methyl groups in aliphatic compounds of high molecular weight by a modification of the Kuhn-Roth method, it was found that the yield of acetic acid varied from 70 to 97% of the theory[13]. A strong oxidising solution and higher temperature (135°C) improved the yields to those mentioned above.

RESULTS: The methyl groups in branched chain fatty acids have been estimated by Ginger[16]. By using his modified method for improving the yield of acetic acid and applying his observations to the dextrorotatory fatty acids of unknown structures isolated from the acetone-soluble fat of tuberculin residues, he concluded that these acids contain doubly branched chains. Campbell and Morton[22] using the same modification have shown the difficulty encountered in obtaining quantitative yields of acetic acid on oxidation of compounds containing gem-dimethyl and tert-butyl groups. The yield of acetic acid has been shown to vary from 62 to 99% per mole of each $-C(CH_3)_3$, $>C(CH_3)_2$ and $\equiv C-CH_3$ group. The method therefore could not be used as a means of determining

the exact number of methyl groups present in a molecule or deciding their particular nature e.g. Lycopene, which contains six $>C-CH_3$ and two $>C<^{CH_3}_{CH_3}$ groups gives acetic acid for six C-methyl groups in 91% yield or for eight groups in 69% yield. As the yield of acetic acid varies considerably and may be as low as 62%, analysis of such compounds will not show whether the acetic acid comes from the $>C-CH_3$ groups alone or from both $>C-CH_3$ and $>C<^{CH_3}_{CH_3}$ groups. Thus it is not possible to correlate the yield of acetic acid with the number of methyl groups present or their particular nature.

The effect of the structure of cyclic compounds on the yields of acetic acid in the determination has been followed by Petru, Jurecek and Kovar[24]. They have observed that the branched cycloparaffins give low yields but cycloolefins, cyclic alcohols and ketones give higher yields of acetic acid. Hence no relation could be established between the structure and the yield of acetic acid.

The difficulty encountered in interpreting the results obtained by the procedure of Campbell and Morton

in some unsaturated straight-chain compounds has been pointed out by Campbell and Chettleburgh[23]. Oleic acid, oleyl alcohol, methyl oleate and linoleic acid which contain only one C-methyl group gave 1.28 to 1.66 moles of acetic acid. These results would be expected from compounds containing two C-methyl groups because the yield in branched chain fatty acids could be as low as 62%[22].

The behaviour of alkylbenzenes towards oxidation in the Kuhn-Roth determination by a modified procedure has been studied by Bradenberger and coworkers[25]. By increasing the concentration of sulphuric acid in the reagent and raising the reaction temperature to 130°C, the interference due to benzoic acid could be avoided as a result of its complete oxidation. The cleavage in the side chain has been established to occur between α and β carbon atoms. Thus ethyl and isopropyl benzene do not yield appreciable amounts of acetic acid but n-propyl benzene gives a good yield. Also, pivalic acid (trimethyl acetic acid) which yields negligible amount of acetic acid under the usual conditions of Kuhn-Roth estimation, gives good yield by this modified procedure. In general, the conditions used have helped in determining the number of methyl groups on the ring

as well as in the side chains having carbon atoms one to twenty, and the results compare well with the expected values.

Eisenbraun et al[26] have shown that the C-methyl determination might be more informative if considered in conjunction with the amount of oxidising agent that is actually consumed by the compound. From the value it should be apparent whether sufficient oxidation has occurred to convert the compound completely to the steam-volatile acetic acid, carbon dioxide and water or if the oxidation had stopped at some intermediate stage with the formation of stable, non-volatile acidic products, e.g. dibasic acids. From the data collected for 69 compounds it has been evident that there is a great variation in the amounts of oxidising agent consumed. The C-methyl value of some of the monobasic acids results from the titration of the unchanged acids. The dibasic acids have not yielded any C-methyl value but show large differences in their resistance to oxidation. The stability of succinic, and to a lesser extent of glutaric and adipic acids to the oxidising agent indicates that these acids as well as acetic acid might be the end products of oxidation.

A careful examination of the results obtained by Eisenbraun *et al*[26] suggests that the quaternary methyl group is more resistant to oxidation than a tertiary methyl group. The yields of acetic acid reported for the relevant compounds are given below:

i)	α,α-Dimethyl succinic acid	0.08 mole
ii)	β,β-Dimethyl glutaric acid	0.01 "
iii)	Methyl isopropyl ketone	1.75 "

Thus the yield of acetic acid is very poor for a gem-dimethyl group and appreciably good for an isopropyl group.

E X P E R I M E N T A L

REAGENTS: Oxidation mixture

Chromic acid-sulphuric acid mixture was prepared by dissolving 16.7 g of chromium trioxide BDH AR in 100 ml of water and adding 25 ml of sulphuric acid AR (sp. gr. 1.84) to it with cooling.

Barium chloride BDH AR

Sodium hydroxide solution 0.01N

MATERIALS: All the samples were of analytical grade. Some of them were readily available in this laboratory. Rest of them were obtained from other sources.

APPARATUS: Thick wall Pyrex tubes having

internal diameter	:	10 mm
external diameter	:	12 mm
length	:	350 mm

Micro Carius furnace with a heating block fabricated to accommodate four tubes and an electronic relay to control the temp. within a narrow range of $\pm 2^{o}C$.

PROCEDURE: About 5-7mgms of the compound was weighed out accurately in a micro carius glass tube. After chilling the

tube in ice cold water, 5 ml of the oxidation mixture was added to the sample. The tube was then sealed at a length of 30 cms and after the carius furnace attained the desired temperature, it was introduced cautiously into one of the pockets of the furnace and the lid properly closed. When the solubility of the compound in the oxidation mixture was found to be somewhat unsatisfactory, the tube was taken out occasionally and turned carefully several times to establish a better contact of the sample with the oxidation mixture. After heating the tube for the desired length of time at the required temperature, it was taken out carefully and placed in a beaker containing water. After chilling the tube with ice-cold water to avoid the loss of acetic acid, the tube was opened as usual and the mixture transferred to the distillation assembly. To ensure a quantitative transfer of the mixture, the tube was washed thrice with 1 ml portions of distilled water and the washings transferred to the distillation assembly. The distillation of acetic acid was then carried out without neutralising the excess chromic acid, and after ascertaining the absence of sulphuric acid with small quantities of barium chloride. The distillate was titrated against 0.01 N sodium hydroxide.

DIFFERENTIAL KUHN-ROTH C-METHYL ESTIMATION

It will be clear from the survey of methods and results of Kuhn-Roth estimation given above, that the gem. dimethyl residue II in a molecule does not contribute significantly to the total acetic acid produced in the oxidation. Furthermore, compounds having one, two or three methyl groups on the same carbon atom are expected, theoretically, to give acetic acid equivalent to one methyl group only, from that part of the molecule.

Since an gem. dimethyl residue in a molecule, ultimately gets degraded to dimethyl succinic or dimethyl malonic acid in the chromic acid oxidation and it is the inertness of these acids to further oxidation which is responsible for the lower acetic acid values in the Kuhn-Roth estimations, it was decided to investigate the Kuhn-Roth estimation of unsym. dimethyl succinic acid. Table I summarizes the data obtained under various conditions.

TABLE I - OXIDATION OF UNSYM.DIMETHYL SUCCINIC ACID

Temperature	Time	Yield of acetic acid
120°	1.5 hrs	0.09 mole
125°	"	0.12 "
130°	"	0.34 "
125°	2.5 hrs	0.14 "
140°	10 hrs	0.54 "

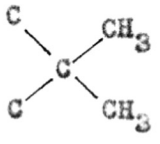
Since it has been found by previous authors that significant losses of acetic acid occur by further oxidation[14] if estimation is carried out at higher temperature, the loss of acetic acid by further oxidation at higher temperature was experimentally determined.

TABLE II - OXIDATION OF ACETIC ACID

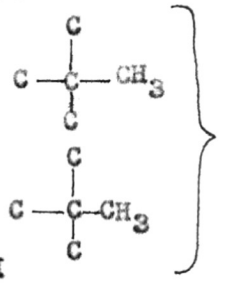
Temperature	Time	% loss of acetic acid
140°	8 hrs	9.5
"	16 "	20.6
"	24 "	30.9

Sesquiterpenoids: From the above data it is clear that unsym. dimethyl succinic acid gives negligible amounts of acetic acid under the usual conditions of Kuhn-Roth estimation (120°C; 1.5 hrs), appreciable quantities of acetic acid (0.54 mole) are produced when the oxidation is carried out at 140°C (10 hrs). This value will appreciate significantly when the loss of acetic acid by further oxidation is also taken into account. Since the evaluation of this loss of acetic acid from a compound of unknown structure will be difficult to determine, it was

argued that the difference in the amount of acetic acid produced under the two conditions - (i) 120°; 1.5 hrs; (ii) 140°; 10 hrs - may provide a measure of the number of gem.. dimethyl groups present, as the estimation of higher temperature is expected to give additional acetic acid arising from quaternary methyl groups only. Furthermore, if the experimental results come up to these expectations then a differentiation between types II and III also



II

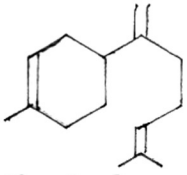
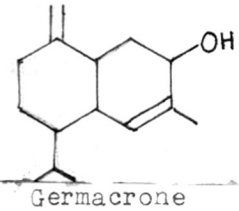
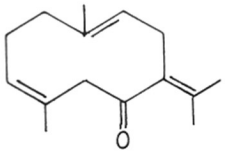
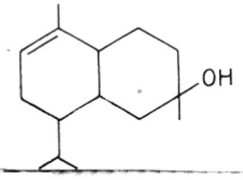


III

becomes possible as the latter should give almost twice the amount of acetic acid.

To test the above reasoning, Kuhn-Roth estimations of a number of sesquiterpenes containing no quaternary carbon, were carried out under the two conditions. As can be seen from the results given in Table III, Δ AcOH is zero or negative, thus conforming to our expectations that in the absence of quaternary methyl groups the usual conditions suffice to furnish all the acetic acid possible from a given structure.

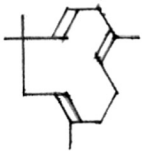
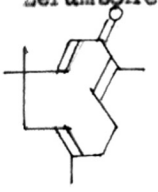
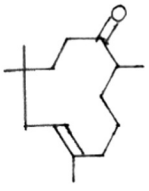
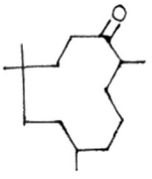
TABLE III - OXIDATION OF COMPOUNDS CONTAINING TERTIARY METHYL GROUP

Compound	Yield of acetic acid in moles		ΔAcOH mole
	120°; 1.5 hrs	140°; 10 hrs	
β-Bisabolene 	2.33	2.22	-0.11
Khusinol 	1.88	1.72	-0.16
	1.86	1.79	-0.07
Germacrone 	2.28	2.29	+0.01
	2.24	2.24	0.00
δ -Cadinol 	2.21	2.13	-0.08
	2.20	2.23	+0.03

Next a series of sesquiterpenoids of known structure and having at least one quaternary methyl group, were subjected to this differential Kuhn-Roth oxidation. Table IV gives the data for various structural types investigated. As can be seen, Δ AcOH is always positive as expected, the average Δ AcOH value per quaternary methyl group being 0.37 mole. By taking this value as equivalent to one quaternary methyl group, the number of quaternary methyl equivalent has been calculated and the results compared with the actual values.

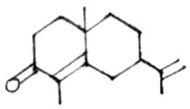
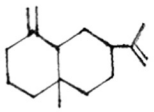
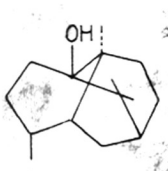
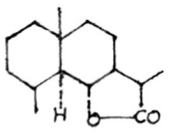
If the gem-dimethyl group (or a quaternary methyl group) is present on a cyclopropane ring, the compound may behave either as belonging to the class of compounds given in Table III (without quaternary methyls) or those in Table IV (with quaternary methyls), depending on the structure of the product on cleavage of the cyclopropane ring by the sulphuric acid present in the reaction mixture. Table V gives the results obtained with two such compounds and as can be seen from their structures, the gem-dimethyl group present on the cyclopropane ring is not expected to contribute to the acetic acid any additional amount at the higher temperature estimation.

TABLE IV - OXIDATION OF COMPOUNDS CONTAINING QUATERNARY METHYL GROUPS

Compound	Yield of acetic acid in mole		△ AcOH mole	No.of quaternary methyl equivalent *	
	120°; 1.5 hrs (i)	140°; 10 hrs (ii)		Found	Actual
Humulene 	1.51	2.01	+0.50	1.3	1
Zerumbone 	1.75	2.15	+0.40	1.1	1
Tetrahydro-zerumbone 	1.64	2.13	+0.49	1.3	1
Hexahydro-zerumbone 	1.60	1.90	+0.30	0.8	1
	1.55	1.92	+0.37	1.0	1

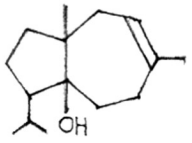
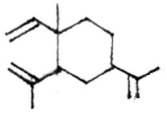
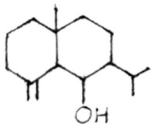
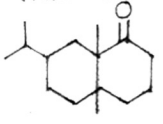
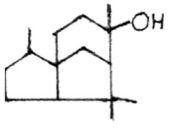
....contd.

TABLE IV (Contd.)

Compound	Yield of acetic acid in mole		ΔAcOH mole	No.of quaternary methyl equivalent*	
	120°; 1.5 hrs (i)	140°; 10 hrs (ii)		Found	Actual
Longifolene 	0.60	1.47	+0.87	2.3	2
Cyperone 	2.02	2.23	+0.21	0.6	1
	2.04	2.20	+0.16	0.4	1
β-Selinene 	1.51	1.71	+0.20	0.5	1
	1.55	1.74	+0.19	0.5	1
	1.47	1.72	+0.25	0.7	1
Patchouli alcohol 	1.31	1.72	+0.41	1.1	1
	1.32	1.73	+0.41	1.1	1
Santanolide C 	1.55	1.97	+0.42	1.1	1

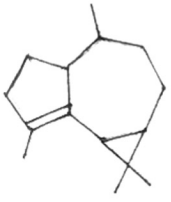
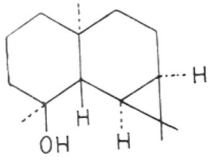
....contd.

TABLE IV (Contd.)

Compound	Yield of acetic acid in mole		Δ AcOH mole	No.of quaternary methyl equivalent *	
	120°; 1.5 hrs (i)	140°; 10 hrs (ii)		Found	Actual
Carotol					
	1.91	2.02	+0.11	0.3	1
	1.89	2.06	+0.17	0.5	1
	1.92	2.14	+0.22	0.6	1
β-Elemene					
	1.41	1.93	+0.52	1.4	1
	1.46	1.99	+0.53	1.4	1
l-Junenol					
	1.10	1.53	+0.43	1.2	1
	1.13	1.54	+0.41	1.1	1
Jatamansone (Valeranone)					
	0.99	1.81	+0.82	2.2	2
	1.02	1.78	+0.76	2.0	2
Cedrol					
	0.89	1.50	+0.61	1.6	1
	0.94	1.51	+0.57	1.5	1

$$\text{*Number of quaternary methyl equivalent} = \frac{\text{moles of AcOH (ii) - (i)}}{\text{average } \Delta \text{ AcOH}}$$

TABLE V - OXIDATION OF COMPOUNDS HAVING QUATERNARY METHYL GROUPS ON A CYCLOPROPANE RING

Compound	Yield of acetic acid in mole		△ AcOH mole	No.of quaternary methyl equivalent *	
	120°; 1.5 hrs	140°; 10 hrs		Found	Actual
α-Curjunene 	2.40	2.22	-0.18	Nil	Nil
Maaliol 	1.73	2.08	+0.30	1	1
	1.71	2.07	+0.36		

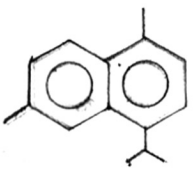
*Quaternary methyls other than those present on the cyclopropane ring.

As can be seen from the above results, differential Kuhn-Roth estimation as described here can be used to determine the number of quaternary methyl groups present. Since a structural residue of the type II is equivalent to one quaternary methyl in the Kuhn-Roth estimation, the method can be used to distinguish whether a compound of unknown structure,

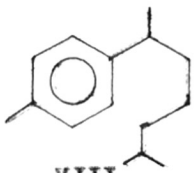
showing two quaternary methyl signals in its PMR spectrum, has these present as an gem.-dimethyl group or two quaternary methyl groups.

As mentioned earlier, the need for a method which could differentiate between the possibilities II and III, arose in connection with the structure determination of sesquiterpenoids himachalenes[8,9] and isolongifolene[10,11]. We discuss below the data obtained with these compounds which helped in giving the necessary information.

The himachalenes are the sesquiterpenes present in the essential oil of Cedrus deodara Loud.[8] These compounds on dehydrogenation with selenium give cadalene and 2-methyl-6(p-tolyl)-heptane as the major products.



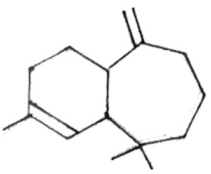
VII



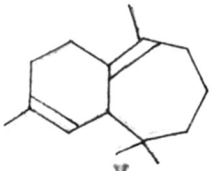
VIII

However, from various considerations it was concluded that himachalenes do not contain an isopropyl group and, in all probability these two methyls are present as an gem. -dimethyl function. Both, α-himachalene and β-himachalene were subjected to differential Kuhn-Roth estimation (Table VI) and the results clearly show that only one

quaternary methyl equivalent is present. These conclusions have since then been completely confirmed as the structures of α-himachalene and β-himachalene are now well established as structures IV and V respectively.

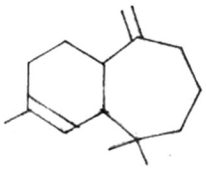
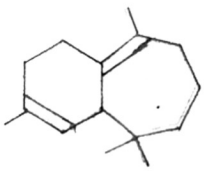


IV

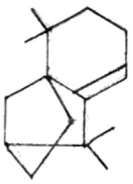


V

TABLE VI - OXIDATION OF HIMACHALENES

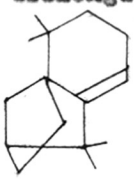
Compound	Yield of acetic acid in moles		△AcOH mole	No. of quaternary methyl equivalent	
	120°; 1.5 hrs	140°; 10 hrs		Found	Actual
α-Himachalene 	1.09	1.56	+0.47	1.3	1
β-Himachalene 	1.58	2.02	+0.44	1.2	1

Isolongifolene is an artefact produced in the acid-catalysed isomerisation of longifolene[10]. At one stage in its structure determination it was necessary to have the information whether in isolongifolene the four quaternary methyl groups (PMR) are present as two units of the type II or as one of this type and two isolated quaternary methyls. Table VII gives the results obtained by differential Kuhn-Roth estimation of isolongifolene. As can be seen, the results clearly show that an equivalent of two quaternary methyl groups is present, and this is in complete accord with the structure VI now known for isolongifolene[11].



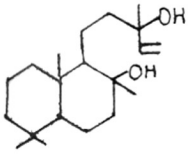
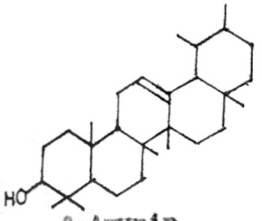
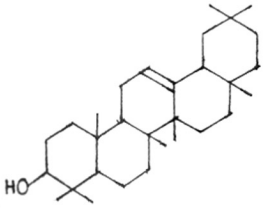
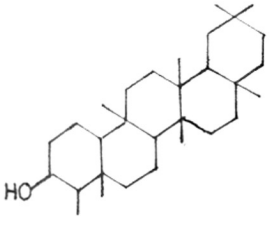
VI

TABLE VII - OXIDATION OF ISOLONGIFOLENE

Compound	Yield of acetic acid in mole.		Δ AcOH mole	No.of quaternary methyl equivalent	
	120°; 1.5 hrs	140° 10 hrs		Found	Actual
Isolongifolene 	0.76	1.58	+0.82	2.2	2.0

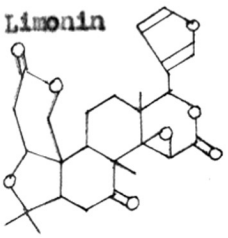
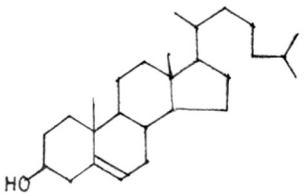
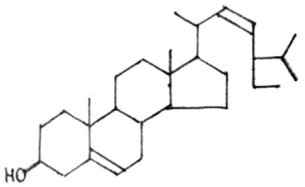
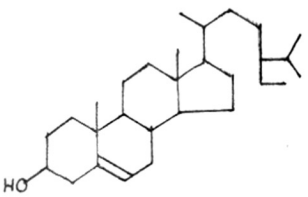
Other Compounds. In order to examine further the scope of this estimation the method was applied to a number of di- and triterpenes and sterols. The results have been collected in Table VIII. It can be at once noted that though limonin contains an equivalent of three quaternary methyls, Δ AcOH is almost zero, indicating thereby that all the possible acetic acid was produced at the lower temperature itself. The most likely explanation for this appears to be high level of oxidation of the molecule itself, especially around the quaternary methyl functions. In all other cases, the Δ AcOH is positive, and using the average Δ AcOH value per one quaternary methyl group, as obtained in sesquiterpenoids series, the number of quaternary methyl equivalents found, are reasonably close to those actually present.

TABLE VIII - OXIDATION OF DI- AND TRITERPENES AND STEROLS

Compound	Yield of acetic acid in mole		Δ AcOH mole	No.of quaternary methyl equivalent	
	120°; 1.5 hrs	140°; 10 hrs.		Found	Present
Sclareol	1.90	2.77	+0.87	2.4	2.0
	1.86	2.56	+0.70	1.9	2.0
α-Amyrin					
	1.75	3.45	+1.70	4.6	5.0
β-Amyrin					
	1.48	3.16	1.68	4.5	6.0
	1.58	3.25	1.77	4.8	6.0
Friedelan-3β-ol					
	0.33	3.02	2.69	7.2	6.0
	0.28	3.00	2.72	7.3	6.0

...contd.

TABLE VIII (Contd.)

Compound	Yield of acetic acid in mole		Δ AcOH mole	No.of quaternary methyl equivalent	
	120°; 1.5 hrs	140°; 10 hrs		Found	Present
Limonin 	2.27	2.16	-0.02	-	3.0
	2.24	2.30	+0.06	-	3.0
Cholesterol 	2.21	2.91	+0.70	1.9	2.0
	2.29	2.83	+0.54	1.5	2.0
Stigmasterol 	2.91	3.48	+0.57	1.5	2.0
	2.92	3.54	+0.62	1.7	2.0
β-Sitosterol 	2.77	3.54	+0.77	2.1	2.0

REFERENCES

1 R. Kuhn and H. Roth, Ber. 66B, 1274 (1933).

2 R.R. Hibbard and A.P. Cleaves, Anal. Chem. 21, 486 (1949).

3 A. Evans, R.R. Hibbard and A.S. Powell, Anal. Chem. 23, 1604 (1951).

4 S.H. Hastings, A.T. Watson, R.B. Williams and J.A. Anderson, Jr. Anal. Chem. 24, 612 (1952).

5 L. Henry and G. Ourisson, Bull. Soc. Chim. Fr. 99 (1955).

6 A.V. Iogansen and E.V. Brown, Trudy Komiss, Anal. Khim. Akad. Nauk SSSR 13, 367 (1963).

Ref. Zhur., Khim., 19 G.D.E. 1964, (9), Abstr.No.9G 185.

7 The Infrared spectra of complex molecules, L.J. Bellamy, p.25, 1958 edition, John Wiley and Sons Inc. New York.

8 G.S. Krishna Rao, Sukh Dev and P.C. Guha, J. Ind. Chem. Soc. 29, 721 (1952).

9 T.C. Joseph and Sukhdev, Tet. letters 6, 216 (1961).

10 U.R. Nayak and Sukh Dev, Tetrahedron 8, 42 (1960).

11 J.R. Prahlad, R.Ranganathan, U.R. Nayak, T.S. Santhanakrishnan and Sukh Dev, Tet. letters 8, 417 (1964).

12 W.F. Barthel and F.B. La Forge, Ind. Eng. Chem. Anal. Ed. 16, 434 (1944).

13 W. Kirssen and E. Stenhagen, Acta Chem. Scand. 6, 682(1952).

14 V.H. Tashinian, M.J. Baker and C.W. Koch, Anal. Chem. 28, 1304 (1956).

15 E. Weisenberger, Mikrochemie Ver Mikrochim Acta 33, 51 (1947).

16 L.G. Ginger, J. Biol. Chem. 156, 453 (1944).

17 W. Schöniger, H. Lieb and M.G. El Din Ibrahim, Mikrochim. Acta 96 (1954).

18 E. Weisenberger, Mikrochim. Acta 127 (1954).

19 a) T. Sudo, D. Shimoe and T. Tsujii, Japan Analyst 6[8], 494 (1957); 6[8], 498 (1957).

b) "Quantitative Organic Microanalysis" by F. Pregl, pp.168, 4th Ed. in English (1945) edited by J. Grant, J and A Churchill Ltd., London.

20 T.S. Gore and S.S. Gupte, Mikrochim. Acta 654 (1961).

21 C.F. Garbers, H. Schmid and P. Karrer, Helv. Chim. Acta. 37, 1336 (1954).

22 A.D. Campbell and J.E. Morton, J. Chem. Soc. 1693 (1952).

23 A.D. Campbell and V.J. Chettleburgh, J. Chem. Soc. 1942 (1953).

24 F. Petru, M. Jurecek and J. Kovar, Chem. Listy 45, 300 (1951).

25 S.G. Bradenberger, L.W. Maas and I. Dvoretzky, Anal. Chem. 33, 453 (1961).

26 E.J. Eisenbraun, S.M. McElvain and B.F. Aycock, J. Am. Chem. Soc. 76, 607 (1954).

CHAPTER II

DETERMINATION OF THE NUMBER AND NATURE OF ACETYL GROUPS IN ORGANIC COMPOUNS

DETERMINATION OF THE NUMBER AND NATURE OF ACETYL GROUPS IN ORGANIC COMPOUNDS

The acetyl group determination is one of the most commonly used functional group estimations. While the estimation gives excellent values in many cases, low values, due to incomplete hydrolysis, are obtained with certain types of N-acetyl derivatives[1-4]. Another factor for unsatisfactory results is the poor solubility of some compounds in the reaction medium[2,5-7].

These limitations led several groups of workers[5-19] to work out alternative or modified procedures. While determining the N-acetyl value of certain compounds (vide infra) by the standard recommended procedure[20] which involves base hydrolysis, followed by rapid distillation of the released acid after acidification, unsatisfactory results were obtained in several cases. Use of some of the modifications recommended in the literature[4], did not give results to our satisfaction. In view of this experience it was decided to study this problem in detail and we now report a modified procedure which enables simultaneous determination of O-acetyl and N-acetyl values, gives superior results for a wider range of N-acetyl compounds and is also economical in time.

PREVIOUS WORK

Several modifications in the procedure, recommending a variety of reagents as also alterations in other factors have been suggested with a view to overcome the difficulties encountered in hydrolysing such compounds. Accordingly, the methods for the determination can be classified into two broad classes; one, where the hydrolysis is brought about with alkali and the other, where it is effected with acid. In the case of alkali hydrolysis, the alkaline solution is acidified after the hydrolysis is over to liberate acetic acid, which is distilled off and titrated as usual. The transesterification method is also favoured by some[8-15]; in this method, the liberated acetic acid forms ester with the solvent alcohol, which in turn is distilled off and hydrolysed further to yield the acid by either of the above procedures.

The reagents employed are methanolic and ethanolic sodium hydroxide and potassium hydroxide for alkali hydrolysis. Acid hydrolysis is carried out by using sulphuric acid, p-toluene sulphonic acid, phosphotungstic acid and, less often, phosphoric and hydrochloric acid. The transesterification methods make use of potassium or sodium methylate in anhydrous methanol[10,11,16]. The Kuhn-Roth

oxidation method employing chromic acid-sulphuric acid mixture has also been used to achieve the same objective[14,17]. However, this method would be helpful only for the compounds of known structures, for the methyl groups attached to carbon atoms also yield appreciable amounts of acetic acid. Obviously, the method has a limited application for the purpose and does not lend itself conveniently for compounds of unknown structures.

An approach by Benson and Turner[18] includes the use of acetic anhydride labeled with ^{14}carbon. The sample is acetylated in pyridine or in the presence of labeled sodium acetate as a catalyst. The specific activity of the solution of a weighed amount of the derivative in dimethyl formamide is determined in a flow counter. The number of acetyl groups is obtained by dividing the specific activity of the derivative by one half the value obtained for the specific activity of the acetic anhydride.

The solubility of the sample in the solvent is of vital importance; deviation from this inescapable requirement results in low values. The solvents used for the reagents are methyl alcohol, ethyl alcohol, benzyl alcohol[5], acetone[2], dioxane[6] and pyridine[7]. The recommended use of pyridine for certain very difficultly soluble

compounds like steroids and triterpenoids has compelled the use of alkali-hydrolysis. On the other hand, Torres et al.[19] have preferred saponification of the acetyl derivatives of polyphenols in acid solutions since they oxidise rapidly in alkaline solutions. The free acetic acid was determined conductometrically.

Both the alkalimetric finish as well as the iodimetric finish are used extensively. However, a subtle point raised by Inglis favours alkalimetry[4]; according to him, the low dissociation constant of acetic acid does not permit iodine to be liberated quantitatively in the iodimetry. Some modified procedures describe elimination of the distillation of acetic acid by using potentiometric titrations[19] and ion exchange chromatography[21,22].

Methods for rapid estimation and simultaneous estimation with other groups. The time required for distilling the acetic acid seems to have attracted the attention of many. A number of modifications seem to have been devoted to improve this part of the procedure. The modified rapid distillation stills of Wiesenberger[14], Schöniger et al.[20] and Sudo et al.[23] have provided answers to this problem. Estimation of acetyl and formyl groups in presence of each other has been accomplished by Kan et al.[24]. Simultaneous

estimation of both these groups by a gas chromatographic method, which needs less time and less substance than the conventional micro procedure, has been reported by Spingler and Markert[25]. A method to estimate acetyl and azido groups in presence of each other has been standardised by Messmer and Mlinko[26].

Difficulties encountered: Freudenberg and Dorr[27] experienced difficulties in hydrolysing certain compounds. In the case of acetylglycine, they had to repeat the operation fifteen times to effect complete hydrolysis, which they brought about using p-toluene sulphonic acid. Kuhn and Roth[1] studied the behaviour of different types of compounds in the acetyl group determination and found that certain N-acetylated compounds were resistant to hydrolysis and needed longer hours and stronger reagents to yield the expected theoretical value. Using 1N methanolic alkali and 5N aqueous alkali they found that acetylglycine required 150 minutes for complete hydrolysis as against certain O-acetylated compounds where hydrolysis could be accomplished in only 30 minutes. Elek and Harte[3] have carried out the hydrolysis by their modified procedure using p-toluene sulphonic acid and the iodimetric finish. Although their reported time for acetylglycine is 150 minutes, it had to be extended to about three hours in certain N-acetylated compounds. The rate of hydrolysis

has also been shown to be appreciably affected by certain structural features like steric hindrance. This has been studied by Bryant and Smith[28] in their study of saponification of esters.

In structural diagnosis it often becomes necessary to distinguish between the N-acetyl groups and O-acetyl groups and to determine the exact number of each in the compound under investigation. The widely used method of Kunz and Hudson[29] in sugar chemistry was modified by Wolfrom et al.[15] and transposed to micro scale by Alicino[2]. It has been shown by Alicino that O-acetyl groups are selectively hydrolysed by 0.01 N sodium hydroxide in an acetone solution of a compound in about two hours at room temperature, leaving the N-acetyl groups unattacked. The method, however, does not lend itself conveniently to the analyses of water-insoluble compounds such as sterol acetates, triterpene acetates etc. and also for deeply colored compounds. Free acidic or basic groups and also the linkages which can open on hydrolysis to liberate acetic acid would interfere seriously and the results therefore would need proper interpretation.

The fallacy in the interpretation of the results obtained in the carbohydrate acetates by earlier workers

has been shown by Inglis[4]. His clarification and revised interpretation have been quite convincing. Using the distillation method of Schöniger et al., he observed that the hydrolysis of acetanilide and phenacetin proceeded only to the extent of about 50%, whereas the usual method of distillation gave the theoretical values. However, the results of the O-acetyl compounds were quantitative, irrespective of the method of distillation. It has therefore been concluded that in the usual procedure, the circumstances are fortuitous and the hydrolysis goes to completion during the distillation of acetic acid after acidification. The low value resulting due to the resistant nature of the N-acetyl bond could be improved to the theoretical one by increasing the strength of the reagent i.e. using 5N sodium hydroxide in 33% methanol in place of 1N, 50% methanolic sodium hydroxide.

PRESENT WORK

While using the modified procedure due to Inglis[4], for certain types of compounds which gave us low results with the standard method[20], it was found that most of the triterpene acetates and sterol acetates remained insoluble in Inglis reagent, which led to unsatisfactory results.

While searching for a useful solvent which could be

incorporated in the Inglis reagent it was found that addition of some pyridine led to dissolution of the triterpene acetates and this gave superior results. It was further found that a mixture of 5N aqueous sodium hydroxide and pyridine could be used and methanol could be omitted. Furthermore, it was found that the usual step of removal of solvent before acidification was unnecessary, in this case, thus economising considerably in time. An additional advantage of this reagent is that it gives negligible blank unlike the Inglis reagent, whene the blank value increases considerably on storage.

The above procedure (1), was next tested for certain representative N-acetyl compounds but was found to be unsatisfactory. The procedure was then modified (2) when satisfactory results were obtained for acetanilide and phenacetin but N-acetyl tryptophane gave low results. A third procedure (3) was then worked out which gave satisfactory results with all the three compounds. These results have been summarised in Table I.

TABLE I - N-ACETYL ESTIMATIONS

Reagent: Pyridine 1 ml+ 5N aqueous sodium hydroxide 1 ml

Acidification: 4 ml 50% sulphuric acid.

Procedure	Compound	Time of hydrolysis.	moles of acetyl	
			Found	Actual
1	Acetanilide	40 min	0.90	1.00
			0.90	
			0.90	
	Phenacetin	"	0.53	1.00
	N-acetyl-dl-tryptophane	"	0.24	1.00
2	Acetanilide	40 min. alkaline +20 min. after acidification.	0.99	1.00
			1.00	
	Phenacetin	"	0.95	1.00
			0.96	
	N-Acetyl-dl-tryptophane	"	0.63	1.00
3	Acetanilide	40 min. alkaline +40 min. after acidification.	0.99	1.00
	Phenacetin	"	1.00	1.00
			1.00	
	N-Acetyl-dl-tryptophane	"	1.00	1.00

Thus, based on the experience of mode of hydrolysis of the acetylated compounds, three procedures (A, B and C) were standardised and investigated for a wider range of substrates.

E X P E R I M E N T A L

Reagents: Pyridine Baker's analytical reagent. Methanol E.Merck Reagent grade. Sodium hydroxide solutions 5N and 0.01N. Conc. sulphuric acid (sp. gr. 1.84). Barium chloride BDH AR.

Materials: Most of the compounds investigated were readily available in this laboratory. Acetylglycine[30], N-acetyl diphenylamine[31], N-acetyl cyclohexylamine[32] and N-acetyl piperidine[33] were prepared by the methods reported in the literature.

Apparatus: Hydrolysis was carried out in the usual apparatus for acetyl group determination. Parnas-Wagner modification of the distillation apparatus was used for rapid distillation of acetic acid, the length of the condenser jacket being increased to 40 cms.

Procedures: A Reagent: 4 ml 50% methanolic 1N sodium hydroxide. (1 ml pyridine if necessary).

Time of hydrolysis: 30 min.

Acidification: 1 ml. 30% sulphuric acid.
(after removal of the solvents).

B. Reagent: 1 ml. pyridine and 1 ml 5N aqueous sodium hydroxide.

Time of hydrolysis: 40 min. alkaline + 20 min. after acidification.

Acidification: 4 ml. 50% sulphuric acid.

C. Reagent: 1 ml. pyridine + 1 ml. 5N aqueous sodium hydroxide.

Time of hydrolysis: 40 min. alkaline + 40 min. after acidification.

Acidification: 4 ml. 50% sulphuric acid.

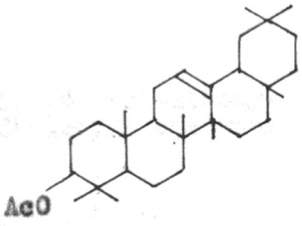
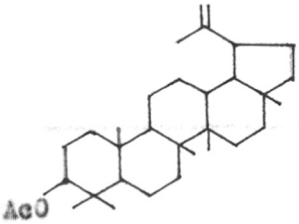
About 5-8 mgm sample was weighed accurately and hydrolysed under the conditions described above. The distillate was collected in four fractions of 50 ml each and titrated against 0.01N sodium hydroxide after checking the absence of sulphuric acid with a small amount of barium chloride.

RESULTS AND DISCUSSION

O-acetyl determination:

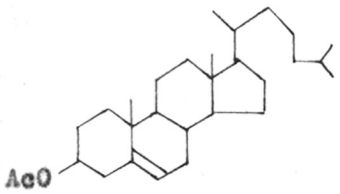
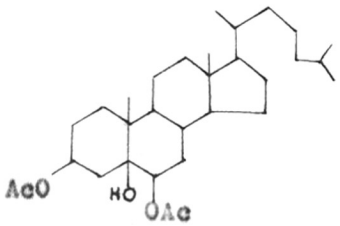
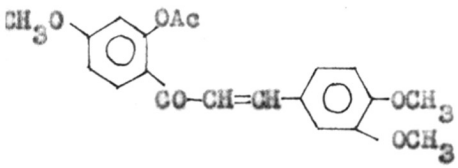
The three procedures (A, B and C) described above were applied to a variety of triterpene compounds and a chalkone acetate. As can be seen from the results given in Table II, all the three procedures gave good results. It may be mentioned here that some of these compounds had earlier given quite unsatisfactory results by using Inglis procedure.

TABLE II - O-ACETYL DETERMINATION

Compound	moles of acetyl Found			Actual
	A	B	C	
β-Amyrin acetate 	1.00	1.02	1.01	1.00
Lupeol acetate 	1.02	1.00	0.99	1.00

.....contd.

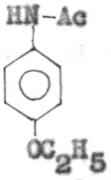
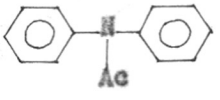
TABLE II (Contd.)

Compound	moles of acetyl			Actual
	Found			
	A	B	C	
Cholesterol acetate 	0.99	1.00	1.01	1.00
3β,6β Diacetoxy cholestan-5α-ol 	1.95	2.00	1.95	2.00
3,4,4'-Trimethoxy-6'-acetyl chalkone 	0.99	0.98	1.02	1.00

N-acetyl determinations

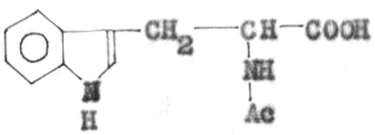
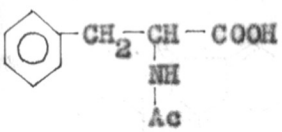
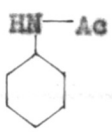
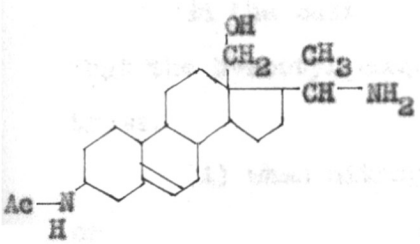
The scope of the above three procedures was next ascertained by examining a number of N-acetyl compounds of considerable structural variety. The results are collected in Table III.

TABLE III - N-ACETYL DETERMINATIONS

Compound	moles of acetyl Found A	B	C	Actual
Acetylglycine $CH_3CO.NH\ CH_2COOH$	0.44	0.99	0.99	1.00
Acetanilide 	0.51	0.99	0.99	1.00
Phenacetin 	0.53	0.97	1.00	1.00
N-Acetyl diphenylamine 	0.43	0.92	0.99	1.00

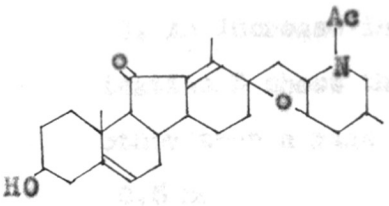
.....contd.

TABLE III (Contd.)

Compound	moles of acetyl Found A	Found B	Found C	Actual
N-Acetyl-dl-tryptophane 	0.13	0.63	1.00	1.00
N-Acetyl-dl-phenylalanine 	0.15	0.72	1.00	1.00
*N-Acetyl cyclohexylamine 	0.10 0.10	0.12 0.13	0.15 0.15	1.00
3,N-Acetyl holarrhimine 	0.11	0.66	0.88 0.87	1.00

.....contd.

TABLE III (Contd.)

Compound	moles of acetyl Found			Actual
	A	B	C	
N-Acetyl piperidine				
	0.79	0.58	0.62	1.00
	0.74	0.54		
N-Acetyl jervine				
	0.50	0.29	0.30	1.00
	0.50	0.30	0.30	

*Results by the conventional procedure were 0.07 mole (2.14%).

On the basis of the above data it would appear that the N-acetyl compounds can be classified into two types.

(i) when nitrogen is a part of a straight chain or is linked to an aromatic ring.

(ii) when nitrogen is attached to an alicyclic ring or is a part of a heterocycle.

It would appear that compounds of class (i) normally give satisfactory results with procedures B or C, while considerably low results are obtained for compounds of class (ii).

From the results described in the Tables II and III, a few generalisations can be drawn:

1. An unchanged value by the above modifications indicated that the group is attached to oxygen.

2. An increase in the yield of acetic acid by the modification B shows that the group is attached to nitrogen atom other than a ring nitrogen. The average increment is $\sim$ 0.5 mole/mole of N-acetyl group.

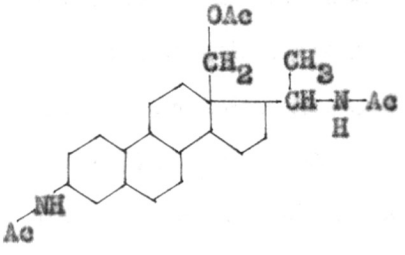
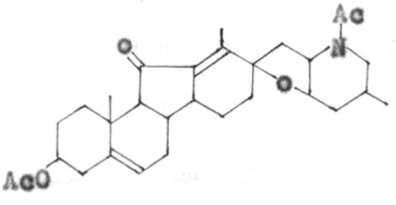
3. A decrease in the yield of acetic acid by the modification B shows that the group is attached to a ring nitrogen atom. The average change is $\sim$ -0.2 mole/mole of N-acetyl group.

4. The groups attached to a ring nitrogen and to a nitrogen atom linked to a saturated ring fail to yield theoretical values by these modifications.

It is thus possible to differentiate between the various types of acetyl groups and to determine the number

of the groups present of each kind by comparing the results obtained under different modifications. Triacetyl holarrhimine and O,N-diacetyl jervine serve as examples for this purpose and the results obtained on them are tabulated in Table IV.

TABLE IV - ACETYL GROUP DETERMINATIONS ON TRIACETYL HOLARRHIMINE AND O,N-DIACETYL JERVINE

Compound	moles of acetyl			
	Found			Actual
	A	B	C	
Triacetyl holarrhimine 	1.36	2.42	2.67	3.00
O,N-Diacetyl Jervine 	1.56 1.60	1.40 1.43	1.38	2.00

Triacetyl holarrhimine: This compound is expected to yield more than one mole of acetic acid by the modification A because it contains an O-acetyl group. As the compound contains two N-acetyl groups, an increase of 1.0 mole is expected when the modification B is used. Also, as one of the N-acetyl groups is linked to a saturated ring, the results obtained even by the modification C should be lower than the theoretical value. The results in the Table IV are in accordance with the expectations showing that the generalisations are borne out.

O,N-diacetyl jervine: As in the case of the earlier compound this compound is also expected to give more than one mole of acetic acid by the modification A, due to the presence of one O-acetyl group. A reduction in the yield of acetic acid by -0.2 mole by the modification B is anticipated due to the acetyl group attached to the ring nitrogen. This situation would also compel the yields obtained by the modification C to be lower than the theoretical value. The results for this compound in Table IV agree with all the expectations, confirming that the generalisations hold good for a complex molecule also.

C O N C L U S I O N

The procedures described above serve as useful

methods for O-acetyl determination of difficultly soluble compounds as well as for certain classes of N-acetyl derivatives. Furthermore, by carrying out the determination by two different procedures (A and B) it is possible, sometimes, to obtain additional information about the nature of acetyl groups present in an unknown molecule.

REFERENCES

1 R. Kuhn and H. Roth, Ber. 66B, 1274 (1933).

2 J.F. Alicino, Anal. Chem. 20, 590 (1948).

3 A. Elek and R.A.Harte, Anal. Chem. 8, 267 (1936).

4 A.S. Inglis, Mikrochim. Acta 228 (1958).

5 S. Sabetay and J. Sivadjian, J. Pharm. Chim. 13,530(1931).

6 F.V. Viditz, Mikrochim. Acta 326 (1937).

7 "Quantitative Organic Microanalysis" by F. Pregl, pp.162, 4th English edition (1945) edited by J. Grant, J and A Churchill Ltd., London.

8 K. Fredenberg and E. Weber, Z. angew. Chem. 38,280(1925).

9 H. Bredereck, Angew.Chem. 45, 241 (1932).

10 R.L. Whistler and A. Jeanes, Ind. Eng. Chem. Anal. Ed. 15, 317 (1943).

11 F.B. Cramer, T.S. Gardner and C.B.Purves, Ind. Eng.Chem. Anal. Ed. 15, 319 (1943).

12 R.G. Stuart, Analyst 72, 235 (1947).

13 J.R. Matchett and J. Levine, Anal. Chem. 13, 98(1941).

14 E. Wiesenberger, Mikrochim. Acta 127 (1954).

15 M.L. Wolfrom, M. Konigsberg and S. Soltzberg, J. Am. Chem. Soc. 58, 490 (1936).

16 L. Mazov and T. Meisel, Analyt. Chim. Acta 20, 131(1959).

17 T.S. Gore and S.S. Gupte, Mikrochim. Acta 486 (1962).

18 R.H. Benson and R.B. Turner, Anal. Chem. 32, 1464(1960).

19 C. Torres, A.S.Capuchino and L. Socias, Anales Soc. espan. fis. quim. 28, 694 (1930).

20 W. Schoinger, H. Lieb and M.G. El Din Ibrahim, Mikrochim. Acta 96 (1954).

21 S. Mizukami and T. Ieki, J. Pharm. Soc. Japan 76, 467 (1956).

22 H. Tani and A. Nara, J. Pharm. Soc. Japan 74, 1399(1954).

23 T. Sudo, D. Shimoe and T. Tsujii, Bunseki Kagaku (Japan Analyst) 6[8], 494 (1957).

24 M. Kan, F. Suzuki and H. Kashiwagi, Microchem. J. 8, 42 (1964).

25 H. Spingler and F. Markert, Mikrochim. Acta, 122 (1959).

26 A. Messmer and S. Mlinko, Acta Chim. Acad. Sci. Hung. 29(1), 119 (1961).

27 K. Freudenberg and K. Soff, Ann. 494, 68 (1932).

28 W.M.D. Bryant and D.M. Smith, J. Am. Chem. Soc. 58, 1014 (1936).

29 A. Kunz and C.S. Hudson, J. Am. Chem. Soc. 48, 1982(1926).

30 R.M. Herbst and D. Shemin, in Organic Syntheses Collective Vol.II, pp.11, Edited by A.H. Blatt 1943 (4th printing 1947), John Wiley and Sons, Inc. New York.

31 A. Claus, Ber. 14, 2366 (1881).

32 A. Baeyer, Ann. 278, 104 (1894).

33 A.W. Hofmann, Ber. 16, 588 (1883).

SOME APPLICATIONS OF SPECTROSCOPIC METHODS OF ANALYSIS

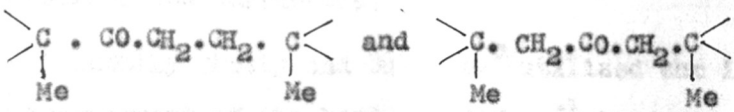
PART II

CHAPTER I
SCISSORING FREQUENCY OF METHYLENE GROUP FLANKING A CARBONYL

SCISSORING FREQUENCY OF METHYLENE GROUP FLANKING A CARBONYL

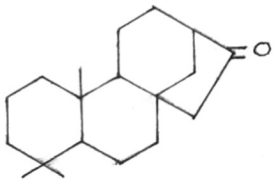
In 1951, Francis[1] reported that the deformation frequency of a methylene group adjacent to a carbonyl occurs around 1410 cm^{-1} i.e. at frequencies considerably lower than those normal (1465 ± 20 cm^{-1}) for methylene without this neighbouring group. This conclusion was confirmed by studies of various deuterated derivatives[2]. Jones and coworkers examined a large number of steroids and found that this is in general true for this series also. However, they found that whereas the scissoring frequency of the methylene group occurs in the range 1408 to 1418 cm^{-1} when the carbonyl is located at 3 or 17 position, the effect is less pronounced when the carbonyl group is present at 4,6,7,11 or 12 positions, the absorption in these cases occuring around 1434 cm^{-1}, close to the position of cyclic methylene groups activated by an olefinic linkage. Noack[3] recently reported δCH_2 for several conjugated cyclohexenones and found the value around 1412 cm^{-1}. The acids containing the grouping $-CH_2COOH$ have also been found by Hadzi and Sheppard[4] to give a strong band in this region; however, complications due to absorption by carboxylic ion in the same region (around 1420 cm^{-1}) can complicate the interpretation.

The above conclusions have been utilized by several authors in determining the presence or absence of a methylene group flanking a carbonyl function in a molecule of unknown structure. Thus Barton and co-workers[5], arguing that the intensity of this band should be proportional to the number of groups contributing to it, could decide between the structural possibilities.

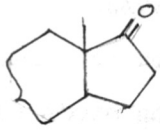


I II

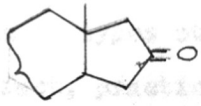
A study of the intensity of the band at 1405 cm^{-1} in the spectrum of the nor-ketone III of phyllocladene was helpful in deciding the position of the carbonyl group[6]. This was done by comparing it with the intensity of the band at 1407 cm^{-1} in the spectrum of 17-oxo-androstan-3β-yl acetate IV which is known to have one α-CH_2 group, and with that of the band at 1410 cm^{-1} in the spectrum of A-norchlorestanone V which has two such groups. The intensity compared with the former one and hence it was concluded that only one α-CH_2 group is present in the nor-ketone III. This could establish



III



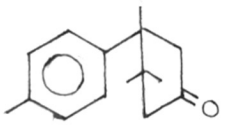
IV



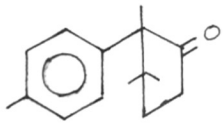
V

the position of the vinyledene group in phyllocladene-precursor of the nor-ketone.

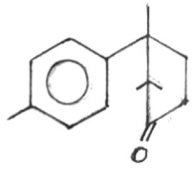
Recently Chetty and Sukh Dev[7] utilized the intensity measurement of the band at 1407 cm^{-1} to decide between the two possible structures VI and VII for β-cuparinone. A comparison of its intensity with that of the α-CH_2 band in the spectrum of α-cuparinone of known structure VIII revealed the presence of two such groups.



VI



VII



VIII

β-Cuparinone was therefore assigned the structure VI.

It can be seen from the few examples cited above that the presence or absence of such a band in the IR spectrum of a molecule, and its intensity measurements can

provide valuable aid in structure determination. However, a survey of literature shows that very little information is available for compounds of types other than those referred to above and, in fact, practically no systematic data on the intensity measurements of this absorption have been reported. The work described in this Chapter has been carried out considering the importance of this absorption as an aid in structure determination. The emphasis has been on the determination of changes in band position and intensity of this absorption with structural variation (ring size and substitution).

E X P E R I M E N T A L

MATERIALS: While most of the compounds of the required purity for this study were readily available in this laboratory, some of them were obtained from other sources.

Carbon tetrachloride and chloroform used for preparing the solutions were of spectral purity.

INSTRUMENTATION: All measurements were carried out in an air conditioned room at 25°C on a Perkin-Elmer 221 spectrophotometer equipped with sodium chloride prism or sodium chloride grating interchange. Standard 937 slit programme was used for recording the spectra. The calibration of the instrument was checked with standard polystyrene before recording. Matched fixed path length cells (0.008 cm) were used to record the spectrum. In order to determine the band area accurately, the frequency scale was expanded four times so that 250 cm^{-1} covered 20 cm.

PROCEDURE: Compounds were generally studied in carbon tetrachloride except when the solubility was unsatisfactory and chloroform had to be used, the concentrations ranging from 0.38 moles to 3.18 moles/litre. Care was taken to see that the optical density varied approximately between

0.3 and 0.6. The intensity measurements were carried out by the method of Wilson and Wells as followed by Ramsay[8] and Jones et al.[9] Thus, the measurements of band area were carried out by determining band heights at approximate frequency intervals of 5 cm^{-1} and the area was calculated using the equation -

$$B = \frac{1}{Cl} \int \log_e \left(\frac{To}{T}\right)_\nu \, d\nu$$

where

B = apparent integrated absorption band intensity in $mole^{-1}$ litre cm^{-2}

C = Concentration in moles/litre

l = path length in cm.

To = intensity of the transmitted radiation

T = intensity of the incident radiation.

STANDARDISATION: Standardisation was carried out using methyl ethyl ketone as the standard and measuring the intensity of the carbonyl band in the region 1700 cm^{-1} for different concentrations varying from 0.04 moles to 0.28 moles.

TABLE I - CARBONYL BAND (1719 cm^{-1}) INTENSITIES IN METHYL ETHYL KETONE

Compound	Intensity B	conc. Mole/litre
Methyl ethyl ketone $CH_3.CO.CH_2.CH_3$	1.53×10^4	0.28
	1.60×10^4	0.20
	1.63×10^4	0.14
	1.60×10^4	0.09
	1.58×10^4	0.05
	1.63×10^4	0.05
	1.71×10^4	0.03
	1.71×10^4	0.17
	1.63×10^4	0.11
Average	1.61×10^4	
Reported[1]	1.68×10^4	

A typical calculation is given below.

Compound : 3-Methyl cyclohexanone

Solvent : Carbon tetrachloride

Concentration : 1.161 moles.

Cell thickness : 0.008 cm

	λ_μ	cm^{-1}	$\frac{To}{T}$ d	$\log_{10} \frac{To}{T}$ d
1	6.94	1441	0.000	0.000
2	6.96	1437	0.081 x 4	0.324
3	6.98	1433	0.234 x 4	0.936
4	7.00	1429	0.296 x 4	1.184
5	7.02	1425	0.275 x 4	1.100
6	7.04	1420	0.182 x 5	0.910
7	7.06	1416	0.075 x 4	0.300
8	7.08	1412	0.027 x 4	0.108
9	7.10	1408	0.000	0.000
			Total	4.862
				x 2.303

$$\int \log_e \left(\frac{To}{T}\right)_\nu d\nu = 11.19$$

$$B = \frac{1}{Cl} \int \log_e \left(\frac{To}{T}\right)_\nu d\nu$$

$$= \frac{1 \times 11.19}{1.161 \times 0.008}$$

$$= 12.05 \times 10^2$$

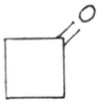
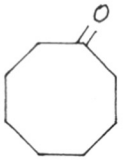
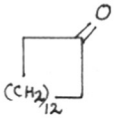
Preparation of 2,6,6-trimethyl cycloheptanone-2,7,7-H_3^2

Methyl deuteralcohol used in this preparation was prepared according to the method of Hobden et al[10].

About 10 mgm of sodium was added to 1.5 ml of methyl deuteralcohol under anhydrous conditions. After the reaction subsided, 0.350 g of tetrahydroeucarvone was injected into it and the solution refluxed for 30 minutes. After cooling the reaction mixture, it was acidified with deuteroacetic acid and diluted with 20 ml water. The deuterated compound was taken up in ether and the etherial layer washed thrice with water and then with aqueous solution of potassium bicarbonate to remove the acid. After washing thoroughly with water, the ether extract was dried over anhydrous sodium sulphate and then evaporated under gentle suction to obtain the desired deuterated product.

The entire process was repeated for complete deuteration and the deuterated compound purified by distillation under vacuum. b.p. 140° (bath temp.) at 170 mm yield, 180 mgms.

TABLE II - INTENSITIES OF THE METHYLENE GROUP FLANKING CARBONYL

Compound	δCH_2CO Intensity B	conc. mole/litre	Figure
$CH_3.CO.CH_2.CH_3$	15.97×10^2	1.00	1(a)
	16.05×10^2	2.24	
	16.82×10^2	1.78	
Average	16.28×10^2		
	17.00×10^2	3.18	1(b)
	18.26×10^2	1.71	
	17.98×10^2	1.37	
	18.42×10^2	1.10	
Average	17.91×10^2		
	9.27×10^2	1.06	1(c)
	9.25×10^2	0.80	
	9.26×10^2	0.84	
	9.18×10^2	1.25	
Average	9.24×10^2		
	10.12×10^2	0.85	1(d)
	9.83×10^2	0.58	
	10.14×10^2	1.58	
Average	10.03×10^2		

.....contd.

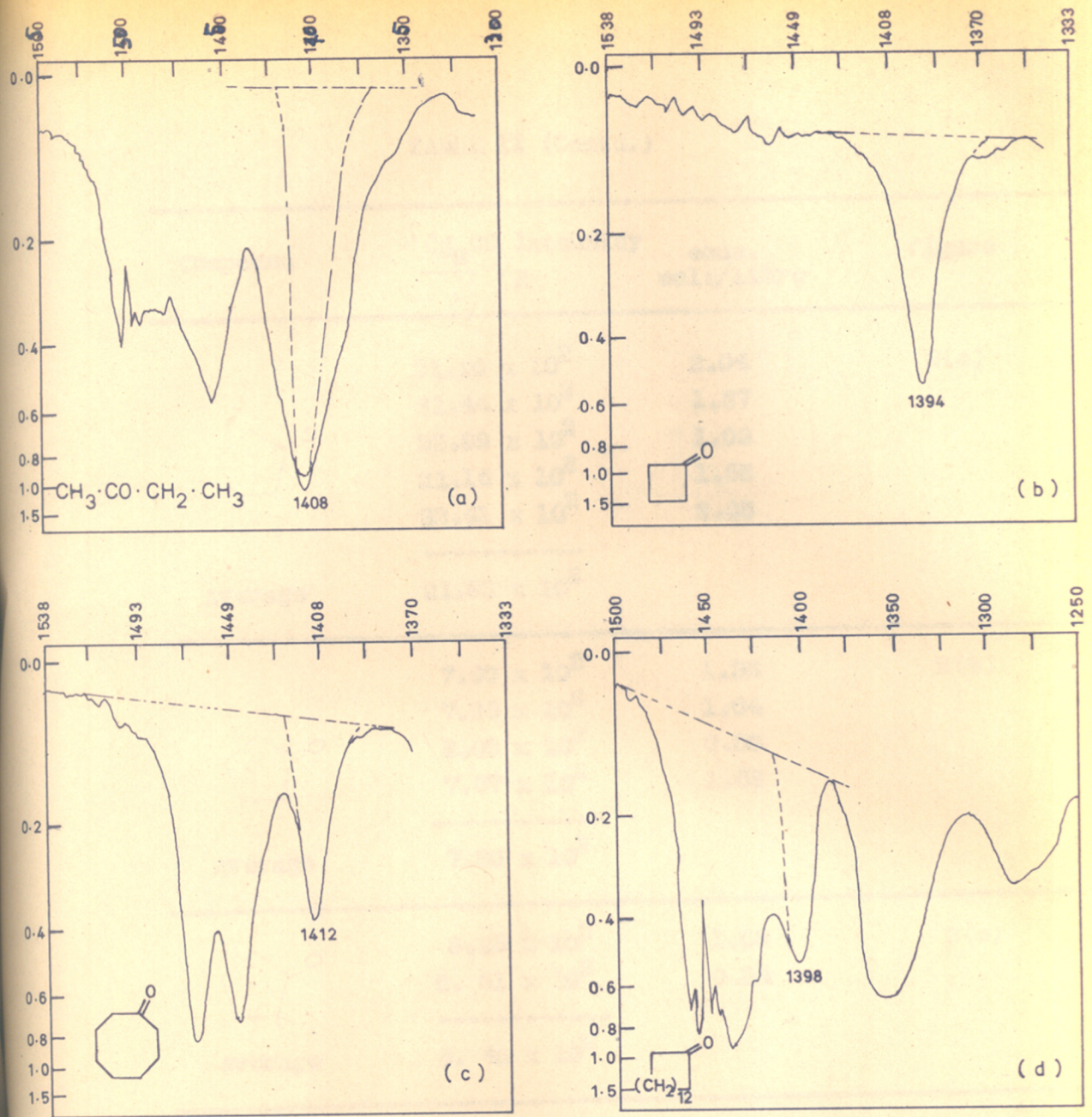


FIG. 1. INFRARED SPECTRA OF —

a) METHYL ETHYL KETONE

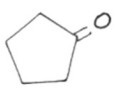
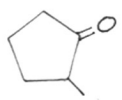
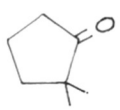
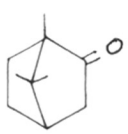
b) CYCLOBUTANONE

c) CYCLO-OCTANONE

d) EXALTONE

IN THE REGION 1540 - 1250 CM^{-1}

TABLE II (Contd.)

Compound	$\delta \underline{CH_2}CO$ Intensity B	conc. mole/litre	Figure
	21.30×10^2	2.04	2(a)
	21.44×10^2	1.37	
	22.89×10^2	1.03	
	21.16×10^2	1.58	
	22.61×10^2	2.08	
Average	21.88×10^2		
	7.60×10^2	1.36	2(b)
	7.95×10^2	1.04	
	8.09×10^2	0.83	
	7.57×10^2	1.58	
Average	7.80×10^2		
	8.25×10^2	1.08	2(c)
	8.61×10^2	0.84	
Average	8.43×10^2		
	8.33×10^2	0.83	2(d)
	7.83×10^2	0.54	
	8.45×10^2	0.50	
	8.69×10^2	1.24	
Average	8.32×10^2		

.....contd.

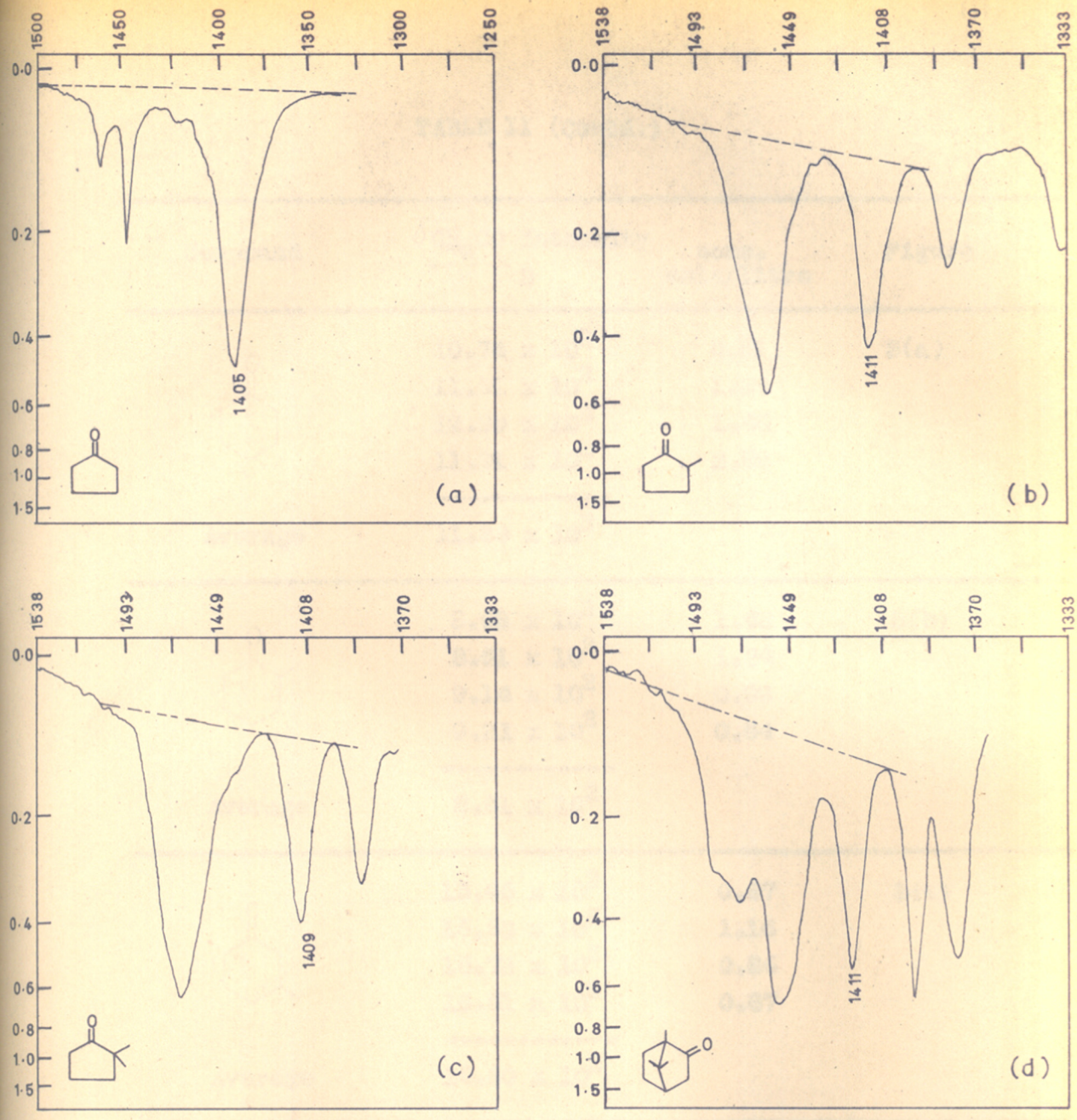
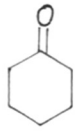
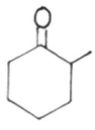
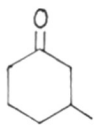
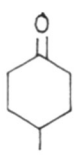


FIG. 2. INFRARED SPECTRA OF —

a) CYCLOPENTANONE
b) 2-METHYL CYCLOPENTANONE
c) 2,2-DIMETHYL CYCLOPENTANONE
d) CAMPHOR

IN THE REGION 1540 - 1250 CM^{-1}

TABLE II (Contd.)

Compound	δCH_2CO Intensity B	conc. mole/litre	Figure
	10.74×10^2	0.61	3(a)
	11.91×10^2	1.29	
	12.99×10^2	1.66	
	11.91×10^2	2.82	
Average	11.89×10^2		
	8.68×10^2	1.62	3(b)
	8.61×10^2	1.34	
	9.14×10^2	0.92	
	9.21×10^2	0.84	
Average	8.91×10^2		
	15.45×10^2	0.67	3(c)
	15.58×10^2	1.16	
	15.72×10^2	0.84	
	16.51×10^2	0.67	
Average	15.80×10^2		
	17.51×10^2	0.38	3(d)
	18.46×10^2	0.51	
	17.79×10^2	0.75	
	17.57×10^2	0.89	
Average	17.83×10^2		

.....Contd.

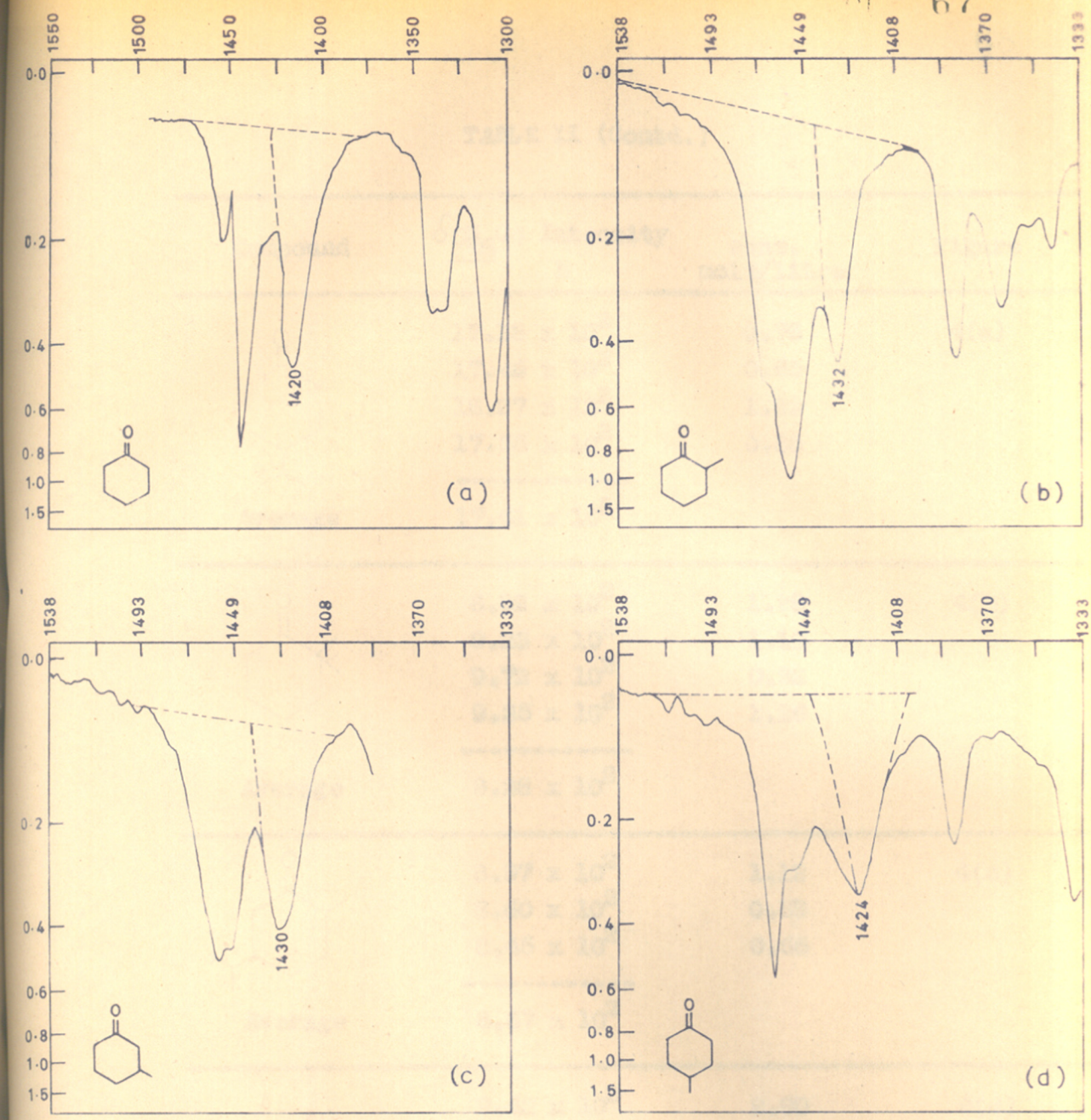


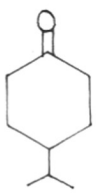
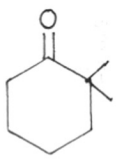
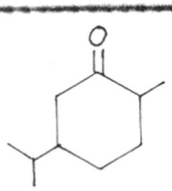
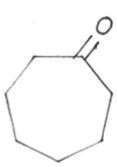
FIG. 3. INFRARED SPECTRA OF —

a) CYCLOHEXANONE
b) 2-METHYL CYCLOHEXANONE
c) 3-METHYL CYCLOHEXANONE
d) 4-METHYL CYCLOHEXANONE *

IN THE REGION 1550-1300 CM^{-1}

* IN $CHCl_3$

TABLE II (Contd.)

Compound	$\delta_{\underline{CH_2}CO}$ Intensity B	conc. mole/litre	Figure
	17.18×10^2	0.76	4(a)
	17.46×10^2	0.65	
	18.27×10^2	1.09	
	17.55×10^2	0.80	
Average	17.61×10^2		
	8.82×10^2	1.28	4(b)
	9.23×10^2	1.10	
	9.72×10^2	0.92	
	9.35×10^2	1.26	
Average	9.28×10^2		
	8.97×10^2	1.12	4(c)
	8.60×10^2	0.88	
	8.45×10^2	0.66	
Average	8.67×10^2		
	9.43×10^2	2.90	4(d)
	10.04×10^2	1.93	
	10.27×10^2	1.26	
	10.77×10^2	1.05	
Average	10.13×10^2		

.....Contd.

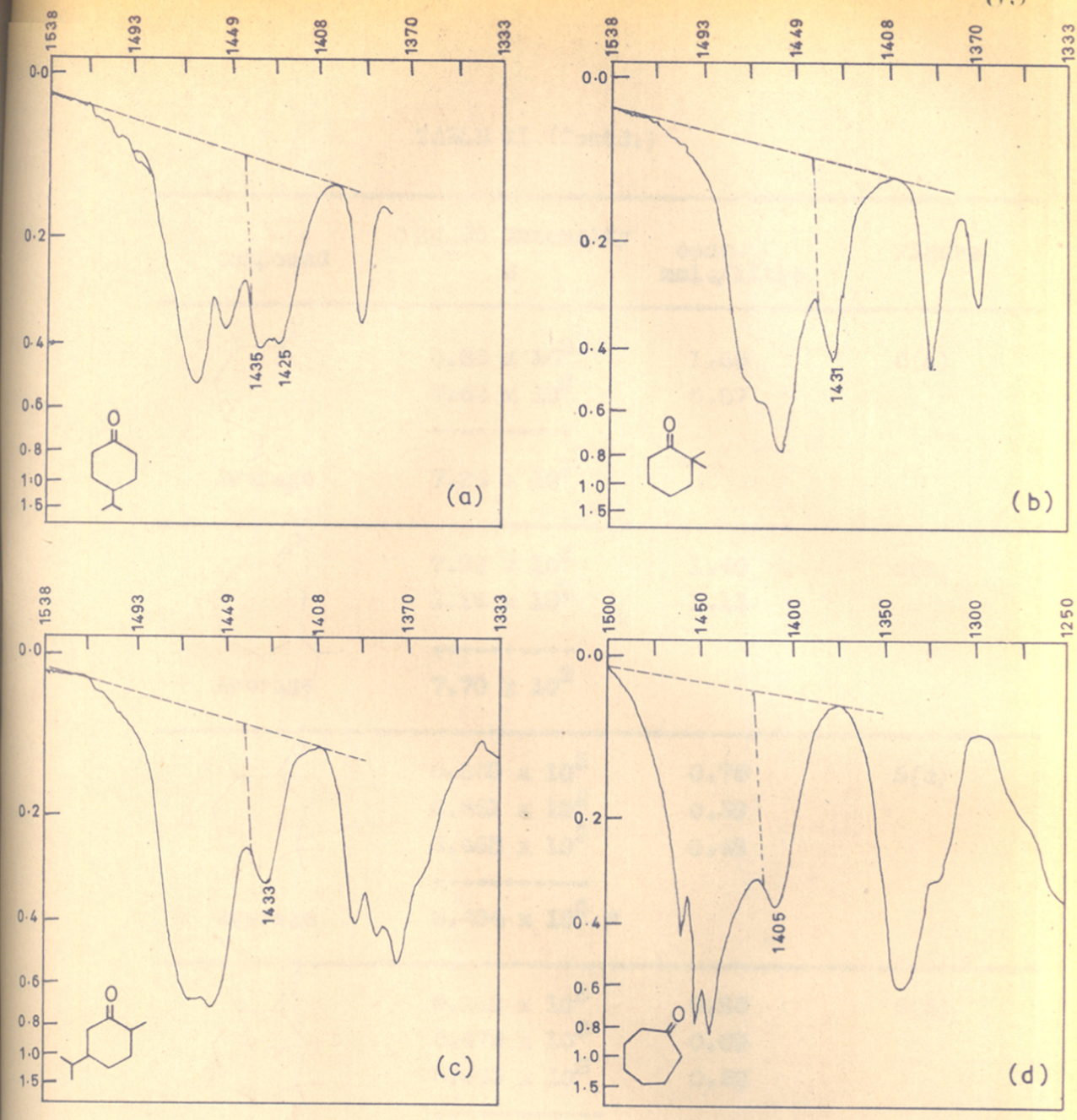
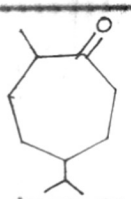
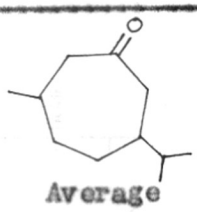
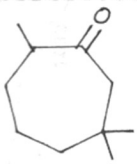
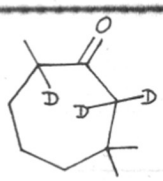


FIG. 4. INFRARED SPECTRA OF —

a) 4- ISOPROPYL CYCLOHEXANONE
b) 2, 2-DIMETHYL CYCLOHEXANONE
c) TETRAHYDRO CARVONE
d) CYCLOHEPTANONE

IN THE REGION 1550- 1250 CM^{-1}

TABLE II (Contd.)

Compound	δ $\underline{CH_2}CO$ Intensity B	conc. mole/litre	Figure
	6.86×10^2	1.66	5(a)
	7.62×10^2	0.87	
Average	7.24×10^2		
	7.22×10^2	1.40	5(b)
	8.18×10^2	1.13	
Average	7.70×10^2		
	6.359×10^3	0.75	5(c)
	6.661×10^3	0.59	
	6.463×10^3	0.48	
Average	6.494×10^3 *		
	5.262×10^3	0.85	5(d)
	5.476×10^3	0.69	
	5.666×10^3	0.52	
Average	5.468×10^3 *		

*Average $\underline{CH_2}CO$ by difference would be 6.494×10^3

(-) 5.468×10^3

(i.e.) 10.26×10^2 $\quad 1.026 \times 10^3$

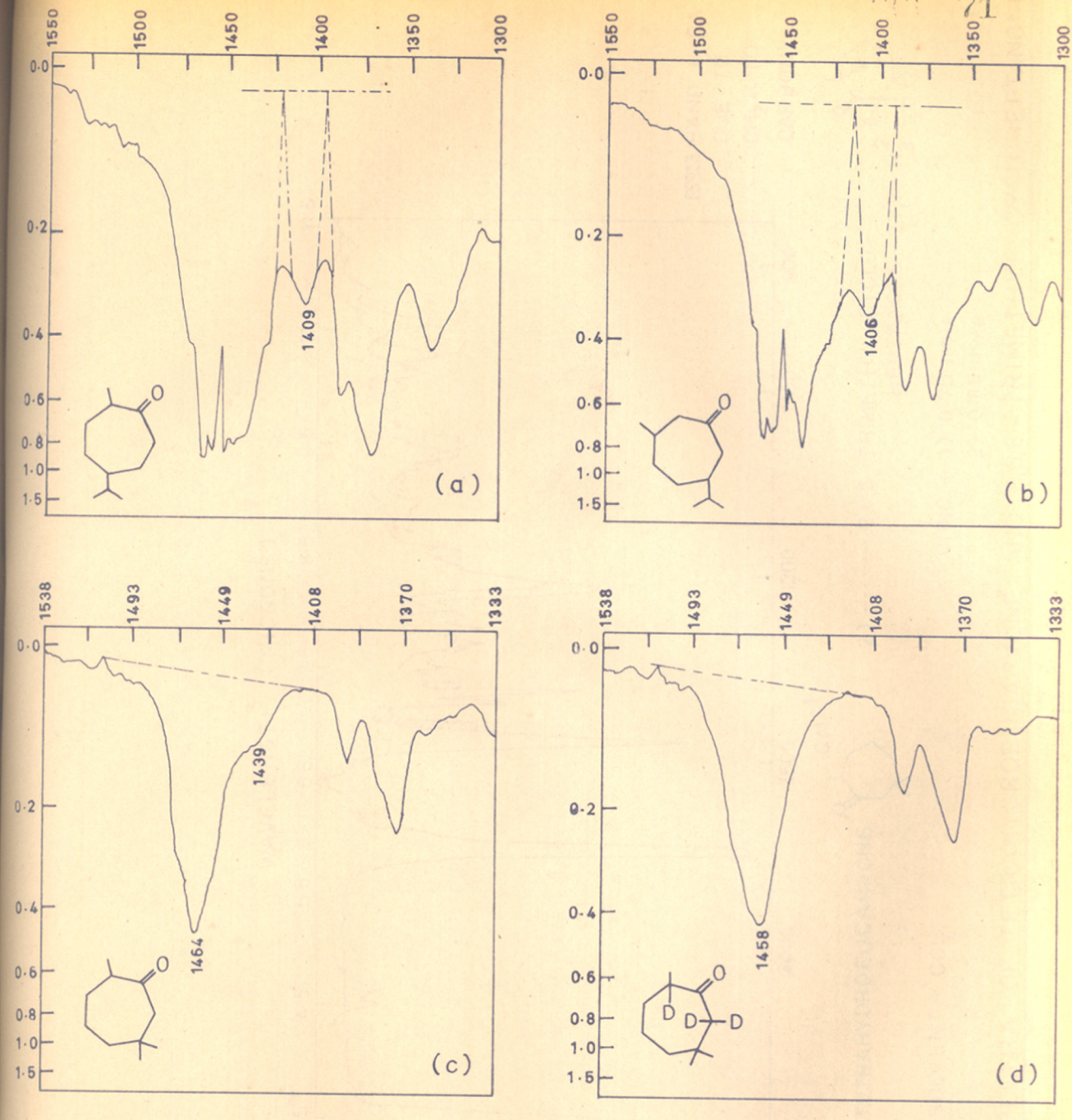


FIG. 5. INFRARED SPECTRA OF —

a) 2-METHYL 5-ISOPROPYL CYCLOHEPTANONE
b) 3-METHYL 6-ISOPROPYL CYCLOHEPTANONE
c) TETRAHYDRO EUCARVONE
d) DEUTERATED TETRAHYDRO EUCARVONE

IN THE REGION 1550-1300 CM^{-1}

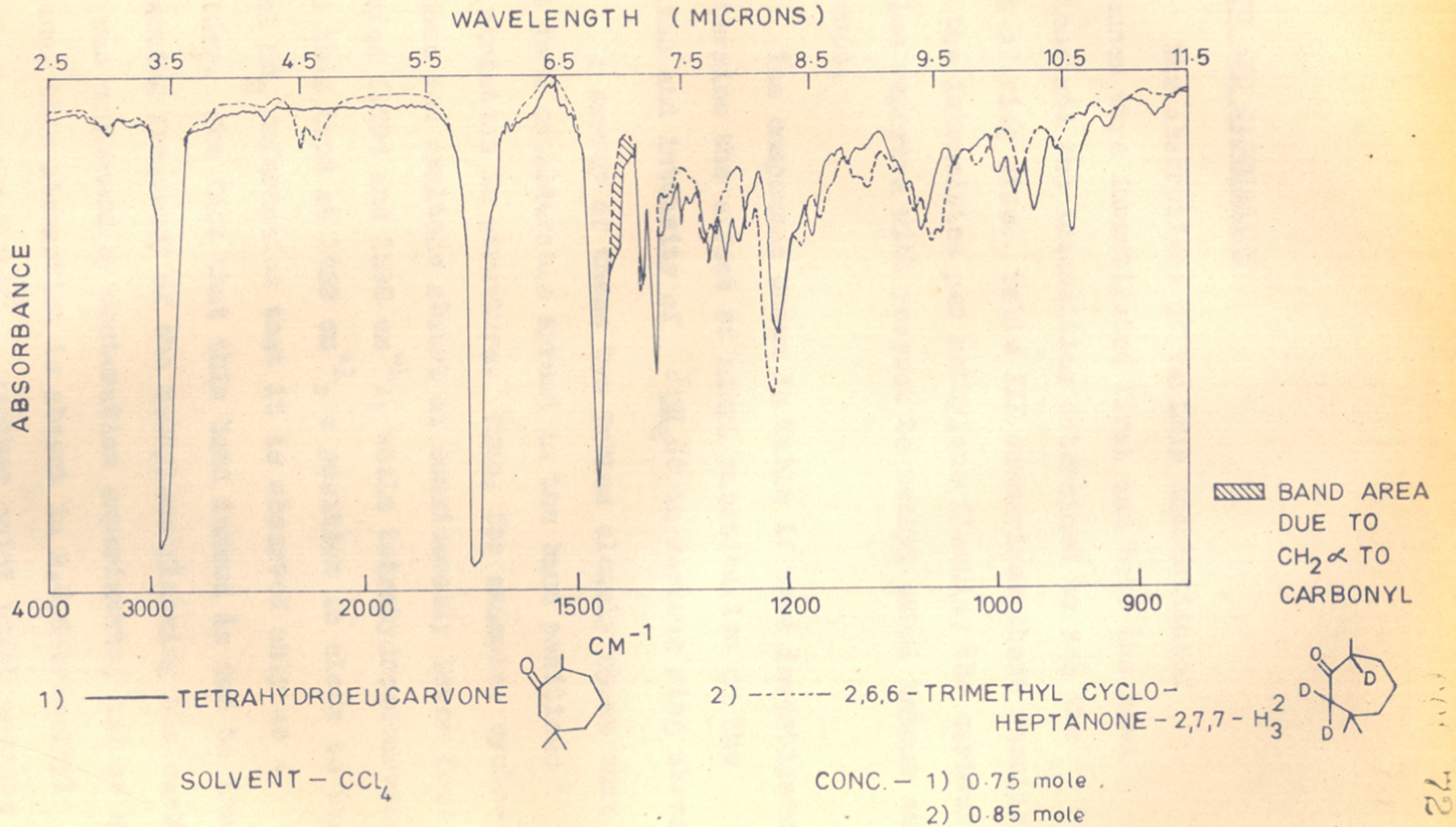


FIG 6 . INFRARED SPECTRA OF TETRAHYDROEUCARVONE AND 2,6,6-TRIMETHYL CYCLOHEPTANONE-2,7,7-H_3^2-

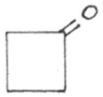
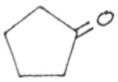
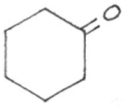
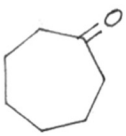
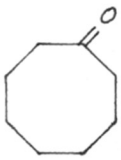
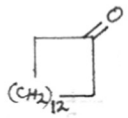
RESULTS AND DISCUSSION

The absorptions of various unsubstituted cyclanones were investigated first and both the band positions and the intensities determined to see the effect of ring size. Table III summarizes these results, where the intensities per methylene flanking the carbonyl are also reported with respect to methyl ethyl ketone as reference.

The compounds shown in Table IV were investigated to determine the effect of alkyl substitution on the position and intensity of δCH_2CO in various ring sizes.

A survey of these two Tables clearly shows that there is a considerable spread in the band positions with variation in structure. Thus, for example, cyclobutanone and exaltone absorb at considerably lower frequencies (1394 and 1398 cm^{-1}), while tetrahydrocucarvone shows this band at 1439 cm^{-1}, a position so close to the normal CH_2 deformation that it is observed only as a shoulder. The fact that this band indeed is due to the scissoring frequency of the methylene flanking the carbonyl, was confirmed by deuteration experiments, and as can be seen, this absorption is absent in 2,6,6-trimethyl cycloheptanone-2,7,7-H_3^2 . Another point which emerges

TABLE III - INTENSITIES OF $\delta \underline{CH_2}CO$ IN UNSUBSTITUTED CYCLANONES PER UNIT METHYLENE GROUP

Compound	$\delta \underline{CH_2}CO$		
	δ max	B/CH_2	Relative B
$CH_3.CO.CH_2CH_3$ (reference)	1408	16.28×10^2	1
	1394	8.95×10^2	0.55
	1405	10.85×10^2	0.67
	1420	5.95×10^2	0.37
	1405	5.07×10^2	0.31
	1412	4.62×10^2	0.28
	1398	5.02×10^2	0.31

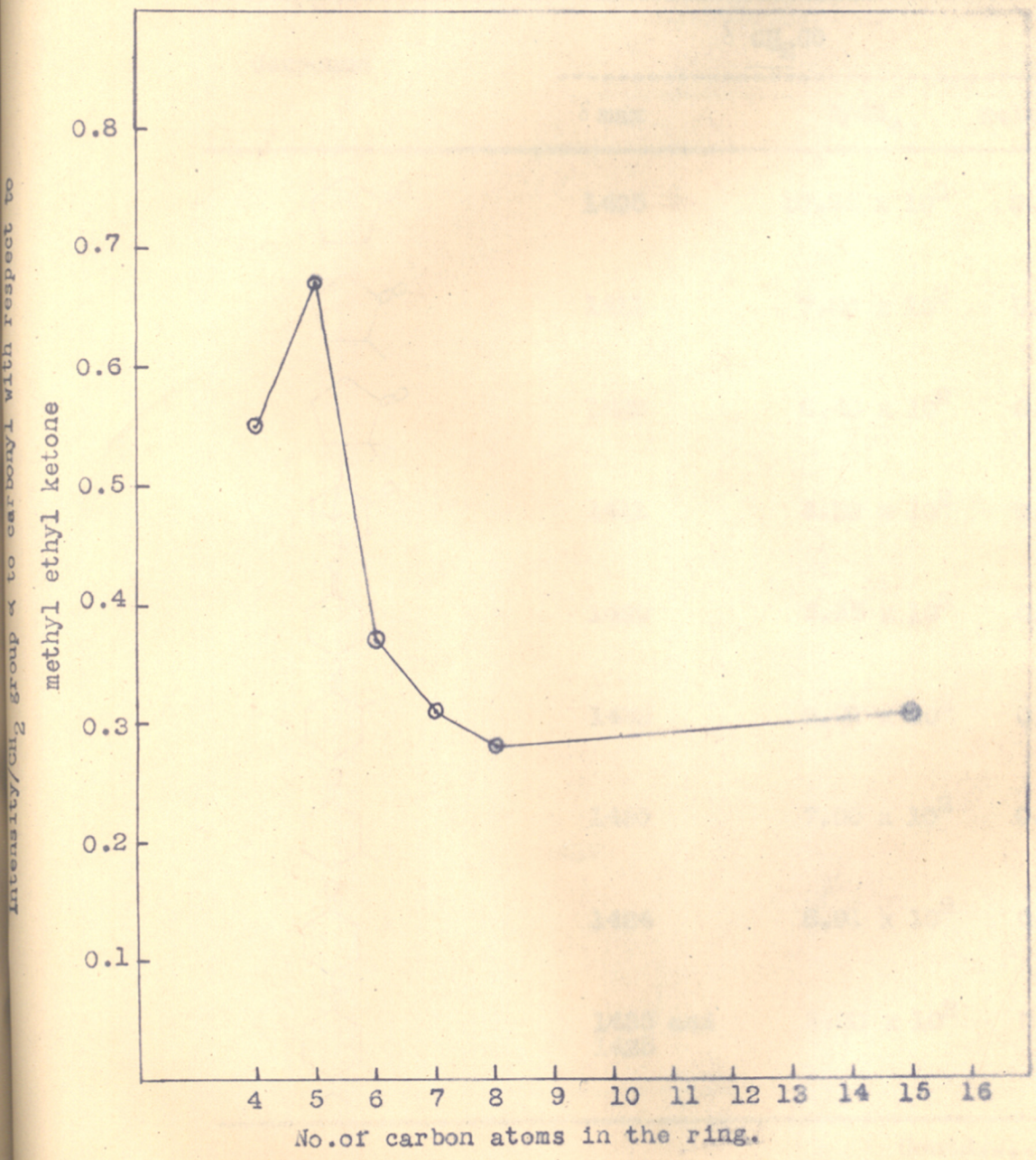
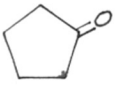
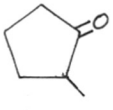
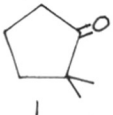
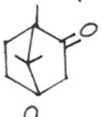
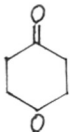
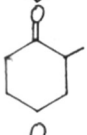
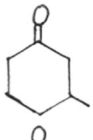
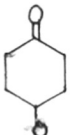
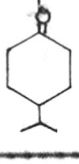


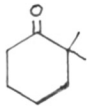
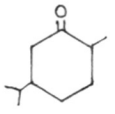
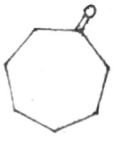
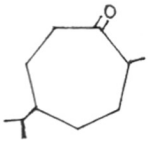
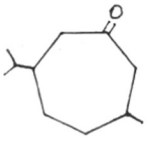
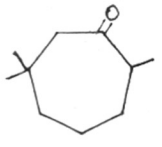
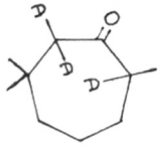
FIG. 7 INTENSITIES OF δCH_2CO IN UNSUBSTITUTED CYCLANONES.

TABLE IV - FREQUENCIES AND INTENSITIES OF δ $\underline{CH_2}CO$ IN CYCLANONES

Compound	δ $\underline{CH_2}CO$		
	δ max	B/CH_2	Relative B
	1405	10.85×10^2	0.67
	1411	7.80×10^2	0.48
	1409	8.43×10^2	0.52
	1411	8.32×10^2	0.51
	1420	5.95×10^2	0.37
	1432	8.91×10^2	0.55
	1430	7.90×10^2	0.49
	1424	8.91×10^2	0.55
	1435 and 1425	8.80×10^2	0.54

Contd.....

TABLE IV (Contd.)

Compound	δCH_2CO		
	δ max	B/CH_2	Relative B
	1431	9.28×10^2	0.57
	1433	8.67×10^2	0.53
	1405	5.07×10^2	0.31
	1409	7.24×10^2	0.44
	1406	3.85×10^2	0.24
	1439	10.26×10^2	0.95
	-		

with some clarity is that the ring size has a definite effect on the position of this band. For example, it can be stated that in general, in cyclopentanones and cycloheptanones this band occurs at around 1405-1410 cm^{-1}, while in cyclohexanones at 1420-1430 cm^{-1}. Furthermore, alkylation at the α position appears to raise the frequency relative to that of the unsubstituted compound by ~ 5 cm^{-1}. These conclusions are clearly seen in Fig.8.

As can be seen there does not appear to be any correlation in the changes in the intensity of this band with variations in the structure. Thus, for example, while the intensity of this band in 2-methyl cyclopentanone goes down with respect to that in cyclopentanone, the intensity of the same band in 2-methyl cyclohexanone goes up with respect to that in cyclohexanone. However, all substituted cyclopentanones and cyclohexanones appear to have a similar relative intensity i.e. ~ 0.5. The intensity variations in the cycloheptanone series appears to be quite random. The intensity of $\delta \underline{CH_2}CO$ in tetrahydroeucarvone, where this band overlaps the normal δCH_2, was obtained by determining the difference between the total integrated intensities for the entire band envelope (Fig.5c and 5d) before and after deuteration.

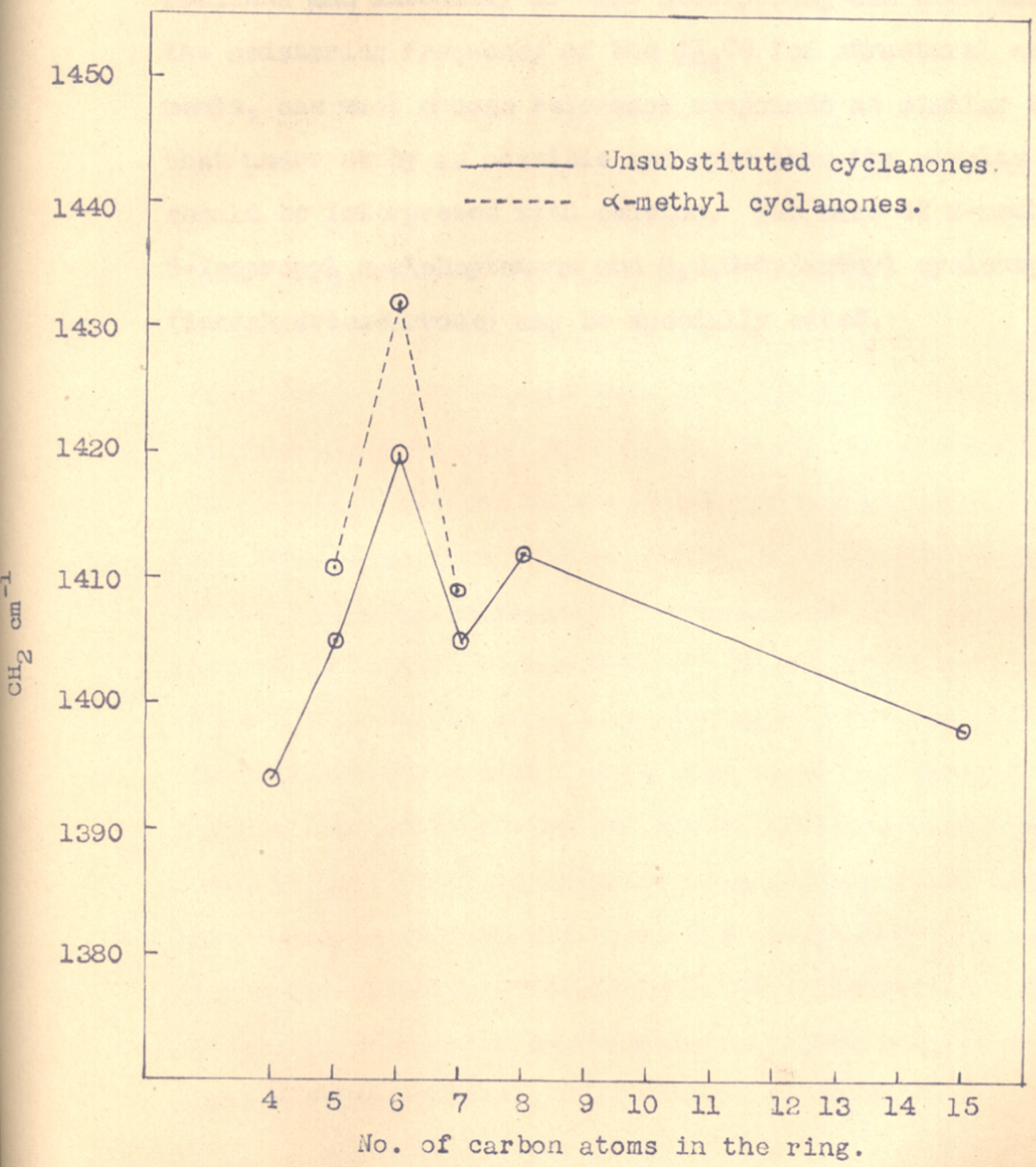


FIG.8 FREQUENCIES OF CH_2CO IN CYCLANONES

From the work described here it becomes clear that variation in structure can have a pronounced effect on the position and intensity of this absorption, and when using the scissoring frequency of the $\underline{CH_2}CO$ for structural assignments, one must choose reference compounds as similar to that under study as possible and even then the results should be interpreted with caution. The case of 2-methyl, 5-isopropyl cycloheptanone and 2,6,6-trimethyl cycloheptanone (tetrahydrocucarvone) may be specially cited.

APPENDIX

SCISSORING FREQUENCY OF METHYLENE GROUP FLANKING A CARBONYL GROUP

Until a few years ago the band intensities of the infra red absorption bands were of minor interest compared to the position of the band. It has been realised only recently that, in so far as the structural diagnosis is concerned, the intensity of a band also has a significant value and that its measurement would be one of the sophisticated means to supplement the information available from the position of the band. However, owing to the inherent shortcomingsin both, the instrument and the method, most of the data collected in the past does not seem to be much useful. Of late, the improvements in the techniques, spectrometers with better resolving powers, corrections for the false scattered radiation and such other factors, have made it possible to determine the intensity parameters more accurately. The data obtained as a result of the measurements can be used for (a) quantitative analysis of mixtures (b) a rather more refined structural diagnosis and (c) calculations of polar properties of bonds and thereby to a better understanding or the chemical reactivity[11].

Methods for measuring the intensity

In the past, the apparent extinction coefficient at a band peak, $E^a_{max} = \frac{1}{Cl} \ln\left\{\frac{To}{T}\right\}_{\nu \text{ max}}$, where

C = Concentration in gm/litre

l = path length in cms

To = intensity of the transmitted radiation

T = intensity of the incident radiation

ν = frequency

has been used as a measure of its intensity. The factors like the finite slit width, slight variations in the spectral resolving power and similar factors concerning the instrument were responsible for the errors in the method and consequently for the difficulties in transfering data from one laboratory for use in another.

The peak height and the band width are dependant upon the slit widths, more so when the latter is comparable in size with the band width; they depend to a certain extent on the optics of the instrument also. When the slit widths become small compared with the half band width $\Delta\nu_{1/2}$, E^a_{max} becomes a much more reproducible and significant quantity, and in certain circumstances is a satisfactory measure of intensity. It is therefore evident that a better resolving power would be helpful and recently the high resolution

double-beam grating spectrophotometers have been used with advantage for a more accurate measurement of the band intensity.

When the solutions obey the well known Beer's law, the <u>true</u> integrated absorption intensity (A) of a band is defined as

$$A = \int \alpha_\nu \, d_\nu = \frac{1}{Cl} \int \log_e \left(\frac{Io}{I}\right)_\nu d_\nu ,$$

where C = concentration, l = path length and I and Io are the intensities of the incident and transmitted monochromatic radiation of frequency ν , and the integral being measured over the limits of the absorption band.

In practice, due to the use of finite slit width, the radiation is not monochromatic and the quantity measured is the <u>apparent</u> integrated absorption intensity (B) which is given by the equation,

$$B = \frac{1}{Cl} \int \log_e \left(\frac{To}{T}\right)_\nu d_\nu$$

where T and To are the intensities of the incident and the transmitted radiation which is not assumed to be monochromatic.

The area under a band should be independent of resolving power[12]. It has been shown experimentally by Ramsay[8] that even when the spectral slit widths are of the

same order as the widths of the absorption bands, the difference in the integrated intensities is only $\sim$ 2-3%, the difference in the peak height due to the finite slit width being roughly compensated by an increase in the band width. However, the true and the apparent molecular extinction coefficients differ by $\sim$ 20%.

Wilson and Wells[13], and Bourgin[14] have shown that errors associated with the use of monmonochromatic radiation could be eliminated by using extrapolation techniques. These involve extrapolation of the apparent band areas to zero concentration or path length, the band areas being measured by graphical integration for various concentrations. Both these methods assume that the incident intensity is constant over the slit-width and the resolving power is constant across the band width. As the application of these methods is quite tedious, attempts were made to develop simple approximation methods to measure band intensities. These methods demand a knowledge of the band shape and involve an assumption that the band profile can be approximated by a mathematical function that can be integrated over the desired frequency range. The approximation by Ramsay[8], which assumes the shape of the band as a Lorentz function, takes into consideration the correction necessary for the band wings. Further, in an attempt to

find out a suitable approximation to determine the band area, he has correlated the apparent peak height $\log_e\left(\frac{To}{T}\right)_\nu$ max with the ratio of the slit width, S, to the apparent half band width $\Delta^a_{\nu 1/2}$ and arrived at the ratios of

$$\log_e\left(\frac{Io}{I}\right)_\nu \text{ max} \Big/ \log_e\left(\frac{To}{T}\right)_\nu \text{ max}$$

i.e. true peak height/apparent peak height and the ratios of

$$\Delta^a_{\nu 1/2} \Big/ \Delta^t_{\nu 1/2}$$

i.e. apparent half band width/true half band width, for a set of values of

$$S/\Delta^a_{\nu 1/2} \quad \text{and} \quad \log_e\left(\frac{To}{T}\right)_\nu \text{ max}$$

Thus the correction necessary to transform the apparent peak height and band width into the true parameters could be known from the tabulated correlation data.

Recently the approximation of Ramsay has been modified by Cabana and Sandorfy[15] and both these methods are widely employed for the measurements of intensity. However, Jones et al.[9] in their critical evaluation of various methods have shown that integrated band area measurements are more satisfactory for both, quantitative determinations of the groups and for the structural diagnosis as well.

The relative significance of extinction coefficients and integrated intensities for quantitative work was considered by Russell and Thompson[16], who have drawn some conclusions regarding the difficulties in the procedures worked out by Ramsay for determining the 'true' band intensities.

Intensity measurement and its correlation with the concentrations of the groups.

Rose[17] determined E^a_{max} of methyl, methylene and methine groups of straight and branched chain paraffins, and naphthenic and aromatic hydrocarbons and established a relation between E^a_{max} and the number of a particular kind of group present. With some exceptions, he could determine the number of such groups in unknown compounds. Fox and Martin[18] carried out measurements on hydrocarbons near 3μ and were able to differentiate between $\gtrless$C-H, $\gtrless\!\!=$C-H, $>CH_2$, $=CH_2$, $-CH_3$. These group types could be characterised by their frequencies and the E^a_{max} could again be correlated with the concentration of a particular type of a group. They found that certain structural features e.g. proximity to a double bond and end methyl groups in long chain hydrocarbons affected E^a_{max} and accurate estimation in such cases was not possible. They

concluded that band area measurements would be more satisfactory for the purpose and used the expression $C \cdot \Delta\nu_{1/2} \cdot E^a_{max}$ to calculate the areas, C having a value close to 1.4. Rose assumed that for an absorption band of a particular group, $\Delta\nu_{1/2}$ the half band width and the frequency of absorption remained unchanged in different molecules. The inadequacy of these assumptions was revealed by Fox and Martin. Cross and Rolfe[19] determined E^a_{max} for the carbonyl group absorption in esters, alkyl and aryl ketones, and saturated, unsaturated and aromatic aldehydes. They found that different classes of compounds can be, to some extent, characterised by E^a_{max}, which are roughly constant within a class, but vary from one class to another, although some irregularities were observed. Using the band area determination method of Wilson and Wells, Francis[1,20] determined the intensities for each of the groups, methyl, methylene and methine in hydrocarbons and esters at their key frequencies to test the equation

$$A_\nu = N_{CH_3} \cdot a_\nu + N_{CH_2} \cdot b_\nu + N_{CH} \cdot C_\nu \ .$$

The agreement was fair but not quite satisfactory because the position of a particular group in the molecule affects the value of A_ν. It was found that the intensities are influenced by the neighbouring carbonyl groups or oxygen atoms.

The carbonyl group absorption bands have been studied in much more details than any other band. The observations of Cross and Rolfe have been confirmed by Hampton and Newell[21] for the carbonyl group in esters. Using the Gaussian function to represent the envelope of the band, the band areas obtained were found in the order of polarity of the carbonyl group by Richards and Burton[22]. The carbonyl stretching mode in esters was found to be twice as big as that in the ketones[1]. The effect of aromatic ring substituents on the frequency and intensity of the carbonyl group in compounds of the type $C_6H_5.CO.R$ was examined by Thompson et al.[23] The shifts of frequency could be correlated with the Hammett factor σ of the substituent group but the intensity of the carbonyl band was almost unaffected. The integrated absorption intensity in compounds of the type $R\,COOC_2H_5$ has been studied by Gutjahr[24], who has discussed the intensity variations with substituents in relation to the inductive and mesomeric effects. Bellanato and Barcelo[25] showed that the intensity values of the carbonyl band in various halogenated acetic acid derivatives were related to their chemical reactivities. An important contribution in this field is due to Barrow[26] who related the intensity of the carbonyl band with the bond polar

properties. He further found a rough proportionality between $\sqrt{A}$ (intensity) and the resonance energy of the carbonyl group, which is a measure of the ionic structures participating in the actual hybrid. It is worth noting that no such correlation exists between the resonance energy and the characteristic group frequency. Jones et al.[9] have studied ketosteroids and found that the intensity of the carbonyl band is controlled by the position of the group on the nucleus or according as there is unsaturation in the ring or presence of halogen atom. They found that when more than one carbonyl group was present, the intensities were almost additive. Jones et al.[27] have studied the band near 1200 cm^{-1} in 3-acetoxy steroids. It has been found that only a single band appears when the hydrogen atom in the position 5 is trans to the 3-substituent, and the band is split when it is cis; studies on intensities and their variations with temperatures suggest the presence of rotational isomers.

Similar studies have been made on the intensities of the bands of other groups like NH[22,28], OH[29], CN[30] and some more functional groups[31]. Flett[32] has determined the intensities of C N stretching bands in nitriles, the C=O stretching bands in esters, ketones, amides, acids

and salts, the N=O stretching bands in nitro compounds and the S=O stretching bands in sulphones, sulphonamides and sulphonic acids. It has been concluded as a result of extensive work that reasonably constant intensity factors could be assigned to several of the above characteristic bands to enable the determination of rough estimates of the concentrations of the respective groups in unknown compounds, provided the total area under the band, rather than the peak extinction coefficient is used as the measure of intensity. Francis[1] has studied the effect of the presence of an adjacent carbonyl group on the intensities of C-H bands in the methyl and methylene groups. It has been observed that the intensity of the O-H and N-H bands is increased but that of C-H band of the methyl and methylene groups in the stretching region is reduced. On the other hand, the deformation band intensity of the methyl and methylene groups is enhanced[33].

Jones and his coworkers have examined the absorption of the methyl and methylene groups in certain steroids and diethyl ketone by selectively deuterating the methyl and methylene groups[2,34,35]. It was proved that in diethyl ketone the methylene groups adjacent to the carbonyl absorb at 1414 and 1355 cm^{-1}. Further, it was observed that the

methylene bending band adjacent to a carbonyl group is commonly observed at 1430 - 1410 cm^{-1}, being displaced down from 1465 cm^{-1} where it occurs in saturated hydrocarbons. Francis[1] had concluded from his intensity measurements that the methylene group α to carbonyl absorbs also between 1355 and 1370 cm^{-1} and the identification of the 1355 cm^{-1} with the methylene group by Jones and his coworkers confirms the conclusion of Francis.

As already indicated earlier it is necessary to know the spectral slit width of the instrument at the particular band frequency to measure the intensity of the band more accurately. This is given by the equation[36]

$$S = \frac{\nu^2 d}{NnF}\left[1 - (n/2\nu d)^2\right]^{1/2}\left\{W_S + \left[\left(\frac{F}{B\nu}\right)^2 + W_A^2\right]^{1/2}\right\}$$

where

ν = Frequency of radiation in cm^{-1}

N = No. of grating passes.

n = Order of spectrum.

F = Focal length of the spectrophotometer

d = Grating spacing

B = Limiting aperture of the spectrophotometer (Mechanical maximum slit width at the particular frequency).

W_S = Mechanical slit width assuming both to be equal.

W_A = Virtual mechanical slit width from the aberration.

The slit widths were calculated for the regions 1700 cm^{-1} and 1420 cm^{-1}, and from the table given by Ramsay[8] it was found that the effect of these small spectral slit widths would be negligible on intensity measurements as they were less than 1/5th of the half band width.

Spectral slit width

at 1700 cm^{-1} (Grating mode)

$$S = \frac{\nu^2 d}{Nnf}\left[1-(n/2\nu d)^2\right]^{1/2} \left\{W_S + \left[\left(\frac{F}{B\nu}\right)^2 + W_A^2\right]^{1/2}\right\}$$

$$= 1.2 \text{ cm}^{-1}$$

at 1420 cm^{-1} (Prism mode)

$$S = \frac{\left[1-n^2\sin^2(\alpha/2)\right]^{1/2}}{4N\sin(\alpha/2)(dn/d\lambda)} \cdot \frac{W}{F} + F(S_s)\frac{1}{2N\,p\,\nu\,(dn/d\alpha)} + S_A$$

where n = refractive index of the prism

α = Apical angle of prism

N = No. of passes

W = mechanical slit width

$F(S_s) = 1$

p = prism base in cms

S_A = Aberration contribution assumed to be zero.

= **1.4 cm^{-1}**

REFERENCES

1 S.A. Francis, J. Chem. Phys. 19, 942 (1951).

2 B. Nolin and R.N. Jones, J. Am. Chem. Soc. 75, 5626(1953).

3 K. Noack, Spec. Acta 18, 697 (1962).

4 D. Hadzi and N. Sheppard, Proc. Roy. Soc. A 216,247(1953).

5 C.S. Barnes, D.H.R. Barton, A.R.H. Cole, J.S.Fawcett and B.R. Thomas, J. Chem. Soc. 573 (1953).

6 W. Bottomley, A.R.H. Cole and D.E.White, J. Chem. Soc. 2624 (1955).

7 G.L.Chetty and Sukh Dev, Tet. letters 73 (1964).

8 D.A. Ramsay, J. Am. Chem. Soc. 74, 72 (1952).

9 R.N.Jones, D.A.Ramsay, D.S. Keir and K. Dobriner, J. Am. Chem. Soc. 74, 80 (1952).

10 F.W. Hobden, E.F. Johnston, L.H.P.Weldon and C.L.Wilson, J. Chem. Soc. 61 (1939).

11 H.W. Thompson, "Report of the conference on molecular spectroscopy" London 1954, The Institute of Petroleum, London, 1955, pp.94.

12 D.M. Dennison, Phys. Rev. 31, 503 (1928).
also see: J.R. Nielsen, V. Thornton and E.B. Dale, Rev. Mod. Phys. 16, 307 (1944).

13 E.B. Wilson, Jr. and A.J. Wells, J. Chem. Phys. 14, 578 (1946).

14 D.G. Bourgin, Phys. Rev. 29, 794 (1927).

15 A. Cabana and C. Sandorfy, Spec. Acta 16, 335 (1960).

16 R.A. Russell and H.W. Thompson, Spec. Acta 9, 133 (1957).

17 F.W. Rose, Jr., J. Research Natl. Bureau Standards 20, 129 (1938).

18 J.J. Fox and A.E. Martin, Proc. Roy. Soc. 175A, 208(1940).

19 L.H. Cross and A.C. Rolfe, Trans. Faraday Soc. 47, 354 (1951).

20 S.A. Francis, J. Chem. Phys. 18, 861 (1950).

21 R.R. Hampton and J.E. Newell, Anal. Chem. 21, 914(1949).

22 R.E. Richards and W.R. Burton, Trans Faraday Soc. 45, 874 (1949).

23 H.W. Thompson, R.W. Needham and D. Jameson, Spec. Acta 9, 208 (1957).

24 L. Gutjahr, Spec. Acta 16, 1209 (1960).

25 J. Bellanato and J.R. Barcelo, Spec. Acta 16, 1333(1960).

26 G.M. Barrow, J. Chem. Phys. 21, 2008 (1953).

27 R.N. Jones, P. Humphries, F.Herling and K.Dobriner, J. Am. Chem. Soc. 73, 3215 (1951).

28 O.R. Wulf and U. Liddel, J. Am. Chem. Soc. 57, 1464(1935).

29 J.J. Fox and A.E. Martin, Proc. Roy. Soc. 162A, 419(1937).

30 Skinner, "Dissertation", Oxford 1954.

31 J.A. Anderson, Jr. and W.D. Seyfried, Anal. Chem. 20, 998 (1948).

32 M.S. T.C. Flett, Spec. Acta 18, 1537 (1962).

33 T.L. Brown, Chem. Rev. 58, 581 (1958).

34 R.N. Jones and A.R.H. Cole [illegible], J.Am.Chem.Soc. 74, 5648 (1952).

35 R.N. Jones, A.R.H. Cole and B. Nolin, J. Am. Chem. Soc. 74, 5662 (1952).

36 K.S. Seshadri and R.N. Jones, Spec. Acta 19, 1013(1963).

CHAPTER II
IDENTIFICATION OF METHYLNAPHTHALENES BY NMR

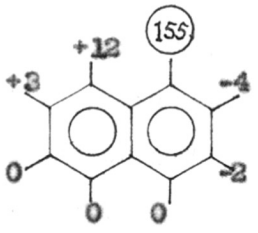
IDENTIFICATION OF METHYL NAPHTHALENES BY NMR

Methyl naphthalenes, have often been obtained as products of dehydrogenation of a variety of terpenoids. The proper identification of these compounds leads to valuable information for the structure of the original material. Unfortunately, however, the yields are usually low and often mixtures of naphthalenes result. These compounds are separated by various chromatographic procedures and very often characterised only by the preparation of suitable molecular complexes, usually the picrates or the TNB (trinitrobenzene) derivatives.

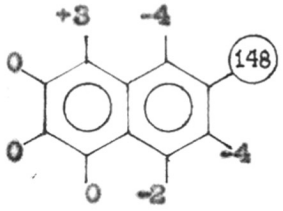
The problem often faced by the worker in this field is the proper identification of these derivatives, as the melting points of many of these compounds are very close[1] and mixed melting points, sometimes, do not lead to a depression. It was thought worthwhile to find out a procedure which would aid in their identification and suggest possible structures for a new compound.

Recently[2] a NMR procedure for the identification of methyl naphthalenes has been reported from these laboratories. A few simple rules have been suggested

which help in the identification of a methyl naphthalene. These rules, which depend on the fact that the position of the methyl signal is governed not only by its position (∝ and β) on the nucleus but also by the disposition of the neighbouring alkyl groups. These rules are summarised in I and II.



I



II

It was thought worthwhile to see how the PMR spectrum of a methyl naphthalene - TNB complex is related to that of the methyl naphthalene, so that, if little change or a predictable shift occurs, the PMR spectrum of the complex could, by itself, serve as an aid to the identification of the methyl naphthalene with the help of the rules worked out for the methyl naphthalenes. Such a procedure would avoid the necessity of regenerating the methyl naphthalene from its complex. Furthermore, the 3H signal due to the three aromatic protons of the TNB moiety, which is expected to be well separated from the aromatic protons of the naphthalene, will act as an internal measure for the methyl signals. The present

Chapter describes the work carried out in this direction.

PMR SPECTRA OF METHYL NAPHTHALENE TNB COMPLEXES

In order to find out a suitable solvent for the TNB adducts, the solubility of TNB and the TNB complex of 1,2,5-trimethyl naphthalene was studied and it was concluded that of CCl_4 and CS_2, the latter is preferable. Hence CS_2 was used as the solvent for all the spectra run during this work. Most of the TNB complexes had a solubility of 1-2% in CS_2 and this sufficed for the measurements.

As expected TNB shows one sharp singlet at 556 c/s. The PMR spectra of TNB complexes of compounds I to VII were taken (Fig.1 to 7) and as can be seen a mere inspection of the spectrum suffices to show the number of methyl groups or any other type of alkyl group present, the TNB protons signal serving as an internal measure for determining the population of the protons. Table I shows a comparison of the signal positions as they occur in the spectra of the TNB complex and that of free methyl naphthalene. As can be seen the position of the methyl signals undergoes little change when measured for the complex. Thus, the empirical rules elaborated[2] for determining the structure of methyl naphthalenes can be

directly applied to the data obtained on the TNB complexes. However, it may be noted that, whereas some of the methyl signals have moved upfield to the extent of 2-4 c/s, the TNB signal undergoes a non-uniform shift.

E X P E R I M E N T A L

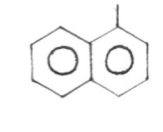
INSTRUMENTATION: All the spectra discussed in this Chapter were recorded on a Varian A-60 High Resolution NMR spectrometer with tetramethylsilane as the internal reference. The values are reported in cycles/sec. from tetramethylsilane as zero.

MATERIALS: All the compounds investigated were available as TNB complexes of authentic purity and were used as such.

Carbon disulphide, reagent grade of E.Merck was used for preparing the solutions.

TABLE I - PMR SIGNAL POSITIONS OF METHYL PROTONS OF FREE METHYL NAPHTHALENES AND THEIR TNB ADDUCTS

Compound	Structure	Positions of signals in c/s				
		Free methyl naphthalene		TNB complex		
		Methyl	Isopropyl	Methyl	Isopropyl	TNB
1-Methyl naphthalene	I	159	-	159	-	560
2-Methyl naphthalene	II	149	-	147	-	558
1,6-Dimethyl naphthalene	III	149, 158	-	148, 156	-	555
1,2,5-Trimethyl naphthalene	IV	144, 150, 156	-	144, 149, 153	-	546
1,3,6-Trimethyl naphthalene	V	144, 147, 154	-	143, 145, 150	-	550
1,6-Dimethyl 4-isopropyl naphthalene (cadalene)	VI	151, 155	77, 85	147, 151	75, 81	554
1-Methyl 7-isopropyl naphthalene (Eudalene)	VII	158	77, 84	156	76, 83	550
Trinitrobenzene	VIII	-	-	-	-	556



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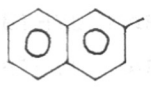

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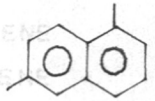

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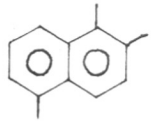

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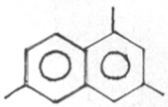

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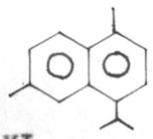

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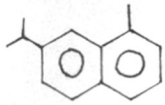

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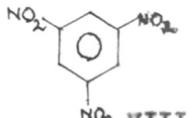


VIII

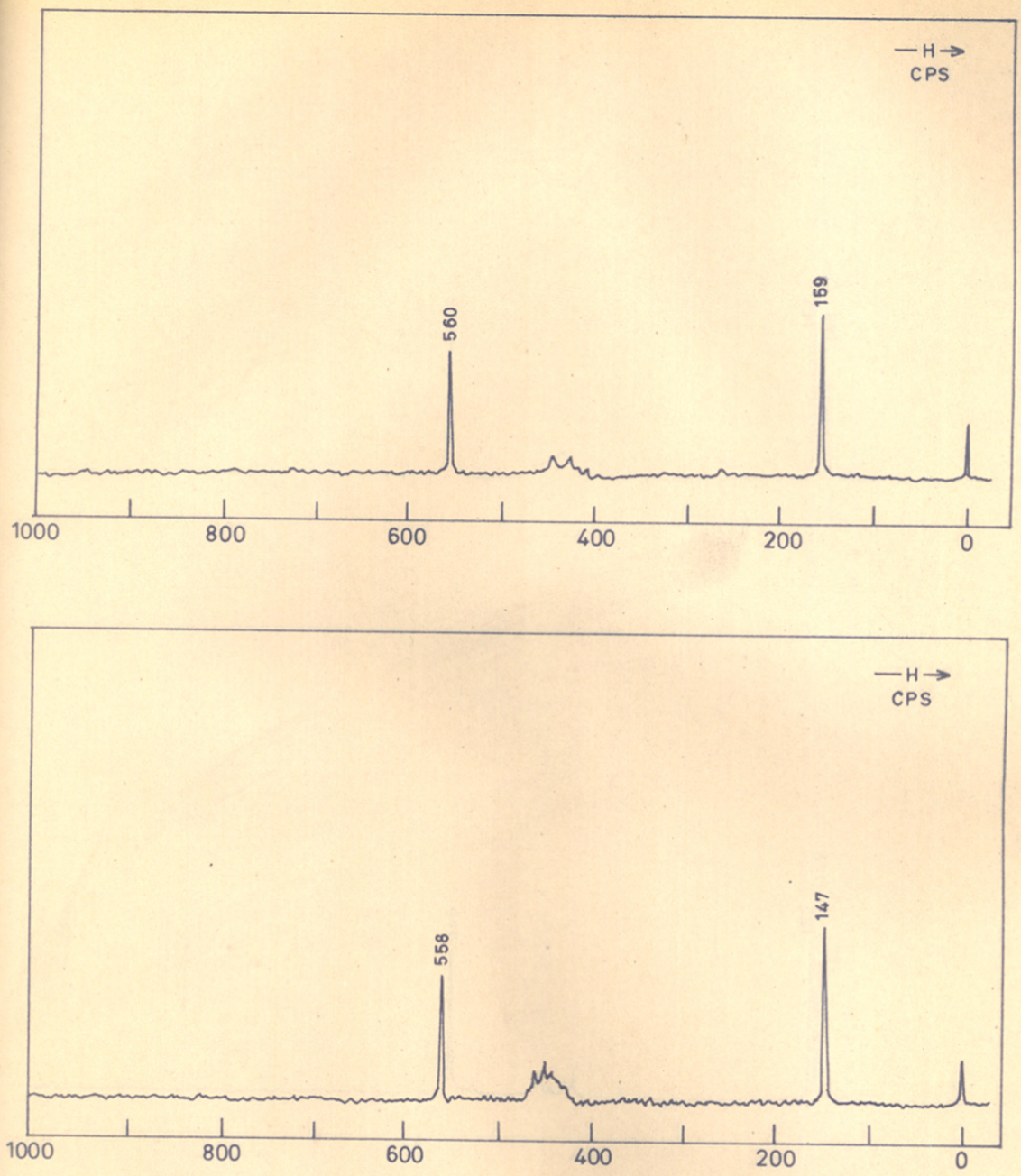


FIG. 1. NMR SPECTRA OF THE TNB ADDUCTS OF

I 1-METHYL NAPHTHALENE

II 2-METHYL NAPHTHALENE

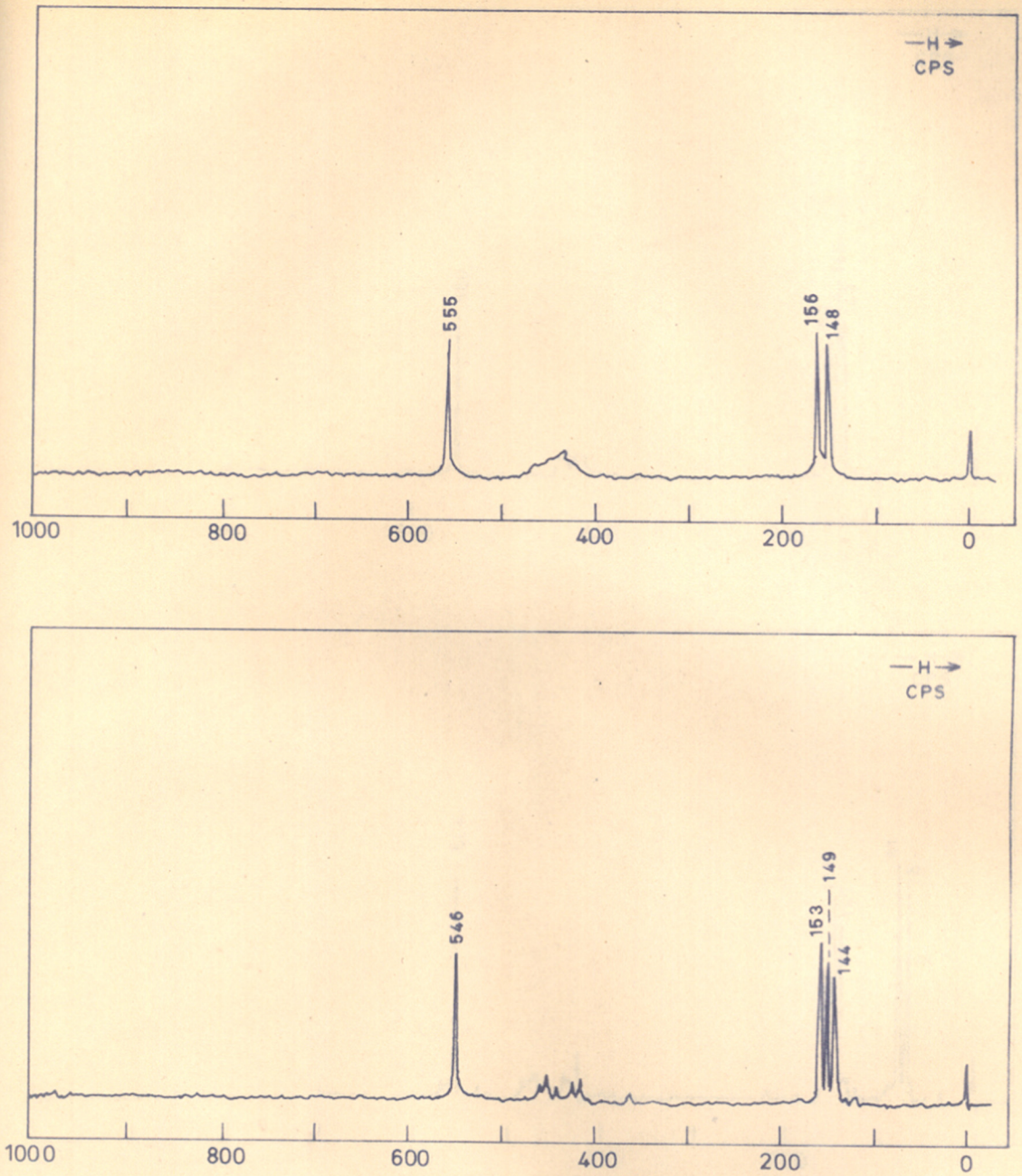


FIG. 2. NMR SPECTRA OF THE TNB ADDUCTS OF

III 1,6-DIMETHYL NAPHTHALENE

IV 1,2,5-TRIMETHYL NAPHTHALENE

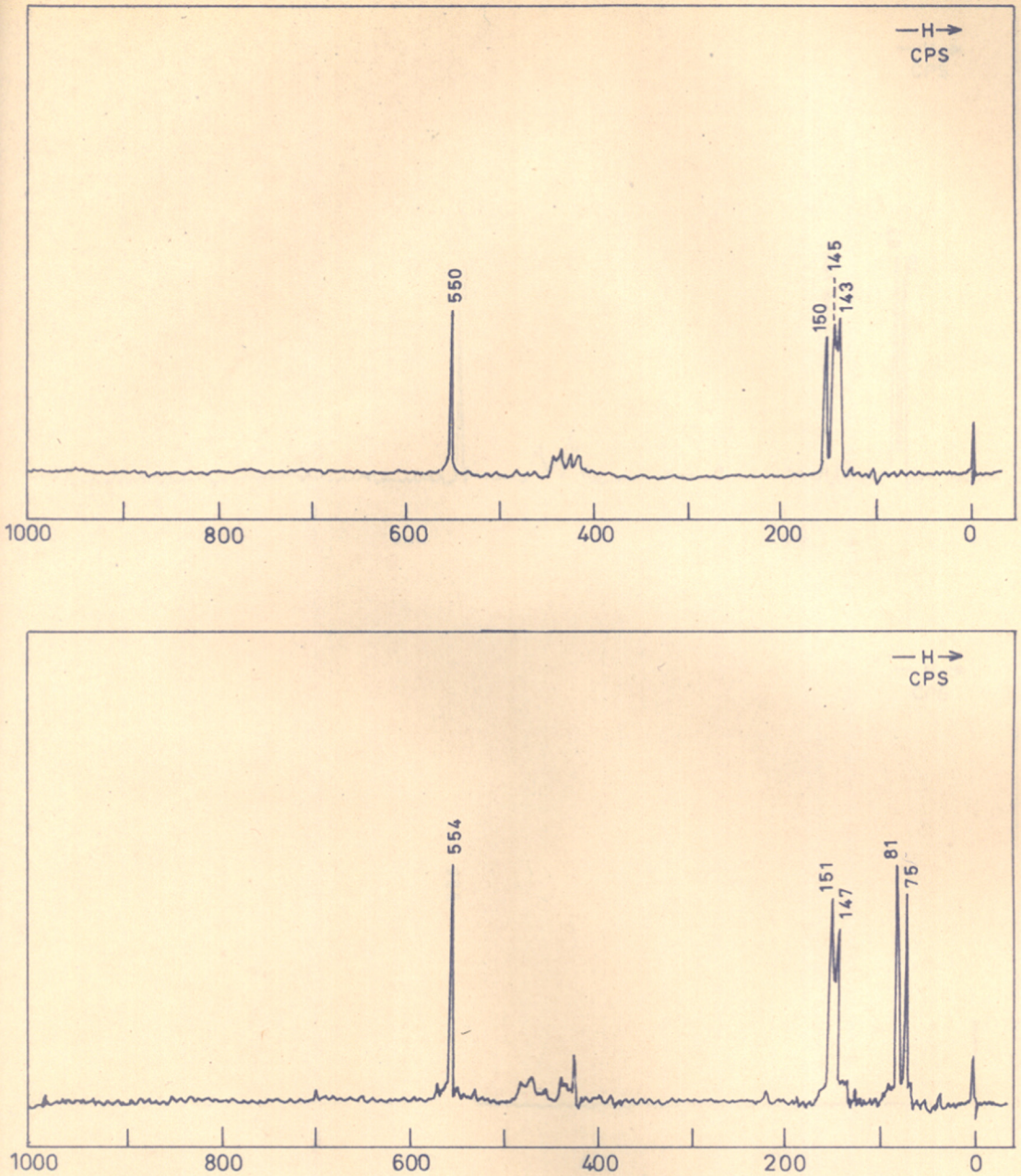


FIG. 3. NMR SPECTRA OF THE TNB ADDUCTS OF

V 1,3,6 - TRIMETHYL NAPHTHALENE.

VI 1,6 - DIMETHYL, 4 - ISOPROPYL NAPHTHALENE

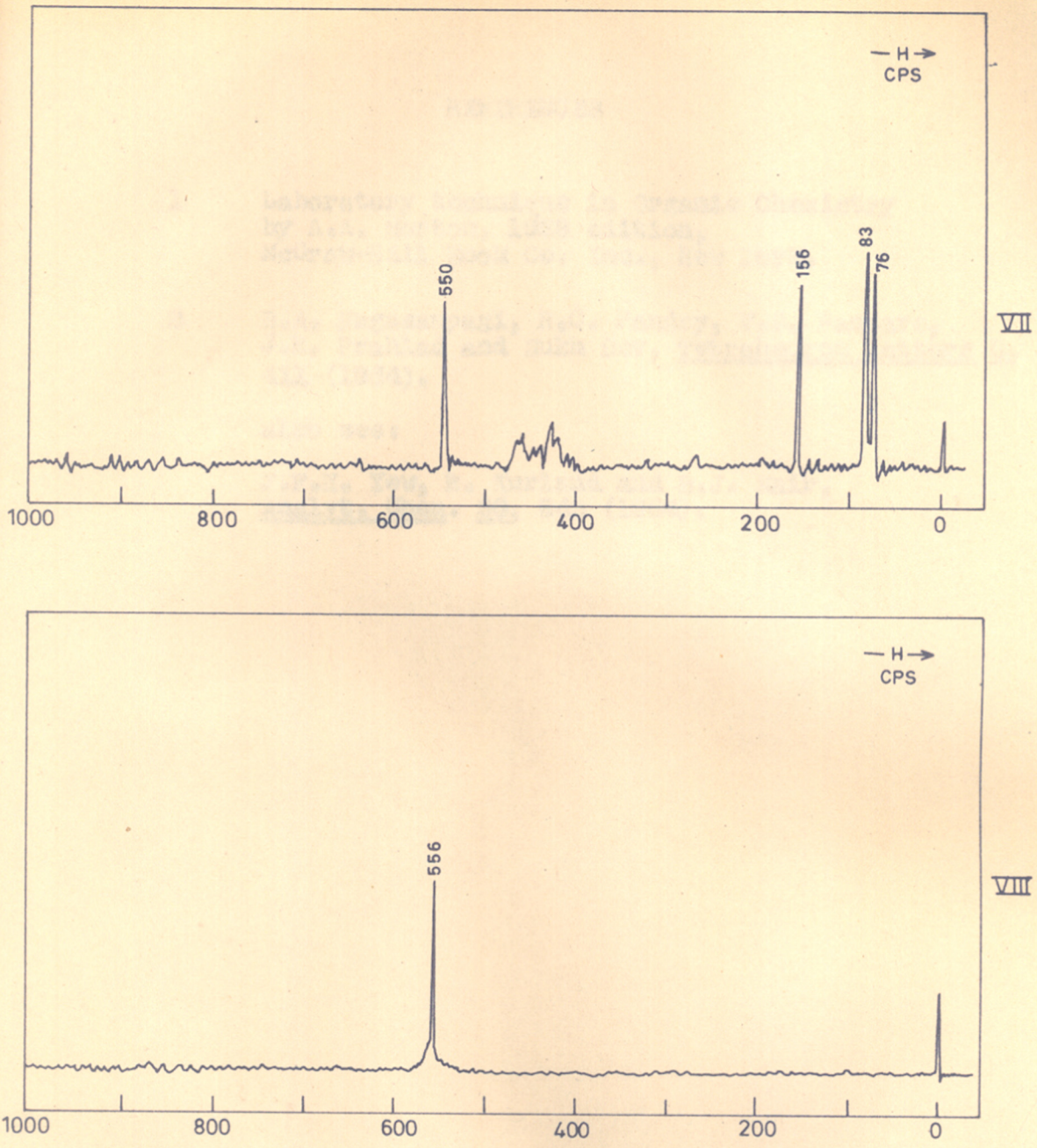


FIG. 4. NMR SPECTRA OF —

VII TNB ADDUCT OF
1- METHYL, 7 — ISOPROPYL NAPHTHALENE

VIII TRINITROBENZENE

REFERENCES

1 Laboratory technique in Organic Chemistry
by A.A. Morton, 1938 edition,
McGraw-Hill Book Co. Inc., New York.

2 B.A. Nagasampagi, R.C. Pandey, V.S. Pansare,
J.R. Prahlad and Sukh Dev, Tetrahedron letters 8,
411 (1964).

also see:

F.F.H. Yew, R. Kurland and B.J. Mair,
Analyt. Chem. 36, 843 (1964).

CHAPTER – III

CHARACTERIZATION OF THE N-METHYL GROUP IN AN ORGANIC COMPOUND BY NMR

CHARACTERIZATION OF THE N-METHYL GROUP IN ORGANIC COMPOUNDS BY NMR

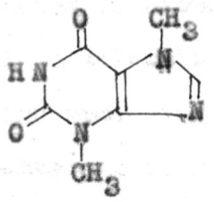
Pregl-Herzig-Meyer method

The microdetermination of methylimino groups in organic compounds is usually carried out by Pregl method[1] which is based on the principle of the macro-method of Herzig and Meyer[2]. The sample on treatment with hydriodic acid forms a quaternary alkyl ammonium iodide which decomposes when subjected to pyrolysis at 350-360°C, splitting off the alkyl iodide. The success of the method depends on the quantitative formation of the quaternary compound and splitting off of the alkyl iodide. Various modifications of the original method of Pregl have been described[3-10].

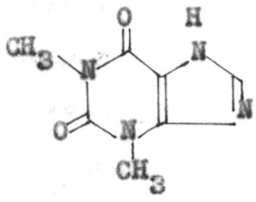
Low values in the estimation have been attributed to the reaction of the alkyl iodide with sodium thio-sulphate solution used in the scrubber to trap the vapours of hydriodic acid[11-14]. It has been shown that methyl iodide dissolves[11] and reacts appreciably with sodium thio-sulphate and ethyl iodide does so to a lesser extent[12]. The reaction of isopropyl iodide with sodium thiosulphate is negligible to be considered for its interference in the

estimation[13]. Several changes suggested to prevent the absorption of methyl iodide by sodium thiosulphate include its substitution by 10% sodium antimonyl tartarate, 5% hydroxylamine hydrochloride, 5% ascorbic acid and 5% hydrazine solution. Sodium thiosulphate dissolved in sodium chloride solution and the use of a mixture of 5% sodium thiosulphate and 5% cadmium sulphate in equal proportions have been recommended by White[11]. Thermal decomposition of the alkyl iodide has been regarded as another source of error by Franzen et al.[7,15]. Compounds having poor solubility could be analysed successfully by Kuhn and Roth[16] using larger proportions of the solvents. However, Haas[17] invariably obtained low results with many alkaloids which he analysed by the Pregl-method.

The variation in the ease with which the alkylimino group can be split off, depends largely on the class to which the compound belongs and the nature of the substituents on the adjacent atoms. This has been beautifully illustrated by Brancone[18] by comparing the results obtained on the isomers, theobromine and theophylline; whereas the former yielded 81% of the theoretical value, the latter failed to yield anything more than 57% of the theoretical value.



Theobromine



Theophylline

Instances have been reported where the N-alkyl group determination gave a positive value inspite of the fact that the group was absent in the compound under test[19].

Application of PMR to N-methyl estimation

In recent years nuclear magnetic resonance spectroscopy has been used for the detection and estimation of N-methyl groups[20,21]. The absorption region for the N-methyl group in the NMR spectroscopy is not quite specific for this particular group. Other functional groups like $N\text{-}COCH_3$, $O\text{-}COCH_3$ and $C\text{-}COCH_3$ sometimes absorb in the same region[20,21], and the identification of the N-methyl signal thus becomes difficult.

Recently, Ma and Warnhoff[21] considering the above difficulties have described an NMR method for the detection, estimation and characterization of an N-methyl group.*

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*This work appeared when our work was in progress.

The method is based on the downfield shift of N-methyl signal in solvents of increasing acidity. The solvents used for recording the spectra are deuterochloroform, perdeuteroacetic acid and trifluoroacetic acid. The downfield shift of the N-methyl resonance is of the order of 0.3 to 1.3 p.p.m. and is dependent on the type of the amine. Most N-methyl groups on neutral nitrogen show little change. For the majority of aliphatic amines in trifluoroacetic acid the NH exchange rate is slow enough at 38° to permit observation of coupling of H(N) with the N-methyl protons, and thus determination of whether the amine is secondary or tertiary. In some cases information can be obtained about methylene and methine groups attached to basic nitrogen.

PRESENT WORK:

The work described in this Chapter was undertaken with very similar objectives, though the stress, in our work, has been essentially on observing the multiplicity of the N-methyl signal, resulting from protonation. This, besides differentiating N-methyl signals from other methyl groups occuring in the same part of the spectrum, is expected to reveal the nature of the N-methyl function, whether it is secondary or tertiary, from the multiplicity of the signal, as in strongly acidic solutions the NH exchange

rate is expected to be slow. Furthermore, it was anticipated that in going from weaker to stronger protonating environments, it should be possible to have some conclusion drawn about the environments of the N-methyl function, as the basicity of nitrogen is severely dependent on various stereoelectronic factors[25].

It has been shown by Grunwald et al.[22] that in methylammonium ion the proton exchange rate between the protons of the ion and water from the solvent medium is appreciably high at high pH (8.56) resulting in lack of coupling of the ammonium protons with the methyl protons. A decrease in the pH (4.01) reduces the exchange rate as indicated by broadening of the methyl group signal, which splits clearly into a quartet at a lower pH (0.96), when the proton exchange is suppressed effectively.

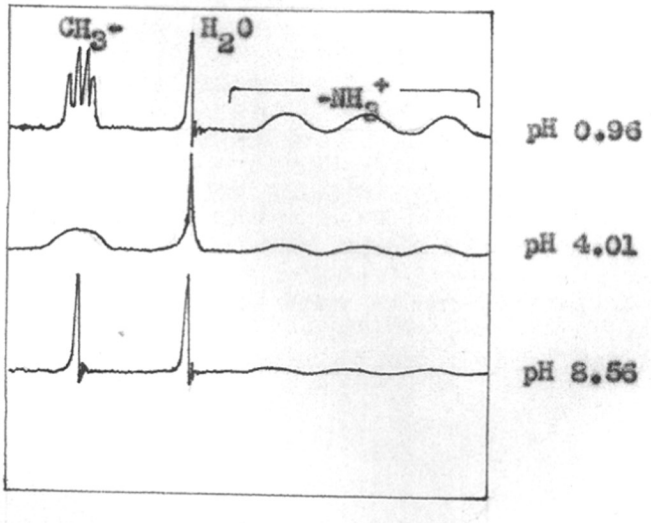


Fig.1

It was therefore argued that N-methyl signal, in NMR spectra of compounds of different basicity, would exhibit such changes in media of varying acidity, as would help in yielding information regarding its identification and characterization.

EXPERIMENTAL

Reagents: Carbon tetrachloride, deuterochloroform and pyridine were distilled and used as solvents after ascertaining their purity. Hydrochloric acid A.R. (sp. gr. 1.18) of Basynth make was used to prepare 1.18N, 1.73N and 3.00N solutions. Perchloric acid A.R. 70% of Riedel-De Haen make was used as such and also diluted to 42%.

Materials: All the compounds used in this work were of the required standard of purity and were readily available in this laboratory, with the exception of N-methyl pyrrolidine, which was prepared by the method reported in the literature[23].

Instrumentation: All spectra were recorded immediately after preparing the solutions (~0.33 molar), on a Varian A-60 high resolution NMR spectrometer with tetramethylsilane as an external reference. The values are reported in cycles/sec. from tetramethylsilane as zero. In the spectra of the aqueous solutions,

the water protons signal was recorded at an amplitude much lower than that for the other signals. The N-methyl signal data obtained on different types of compounds is shown in Table I. (p.141).

DISCUSSION

1. Aliphatic and alicyclic amines: The spectra of some aliphatic and alicyclic amines are given in Fig.1-4. In the case of dimethylamine in water (Fig.1a), the NH proton is undergoing a rapid exchange with water protons[22] and hence is incapable of splitting the N-methyl signal, which appears as a singlet at 147 c/s. In acid medium (Fig.1b), the exchange of NH proton with water protons is effectively suppressed and the secondary N-methyl signal splits into a triplet centred at 179 c/s.

The aqueous solution of trimethylamine (Fig.2a) exhibits a singlet at 156 c/s which changes into a doublet (Fig.2b) centered at 189 c/s, in aqueous hydrochloric acid.

N-methyl pyrrolidine behaves likewise; its signal appearing at 138 c/s (Fig.3a) in CCl_4, is seen as a doublet centred at 187 c/s (Fig.3b) in 1.18N hydrochloric acid medium.

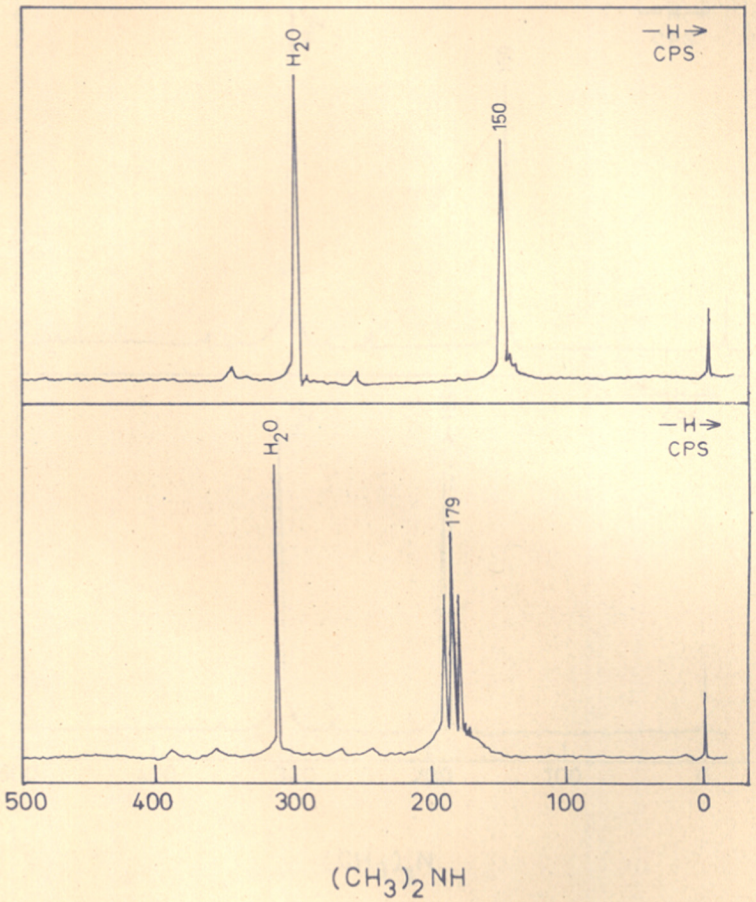


FIG. 1. NMR SPECTRA OF DIMETHYLAMINE.

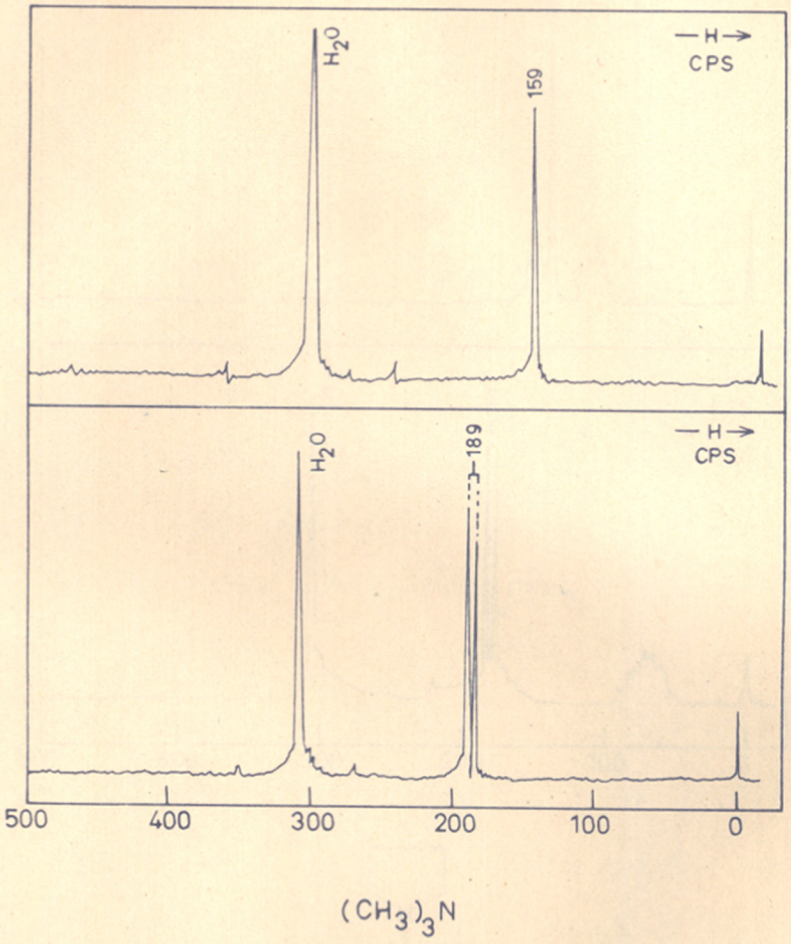


FIG. 2. NMR SPECTRA OF TRIMETHYLAMINE

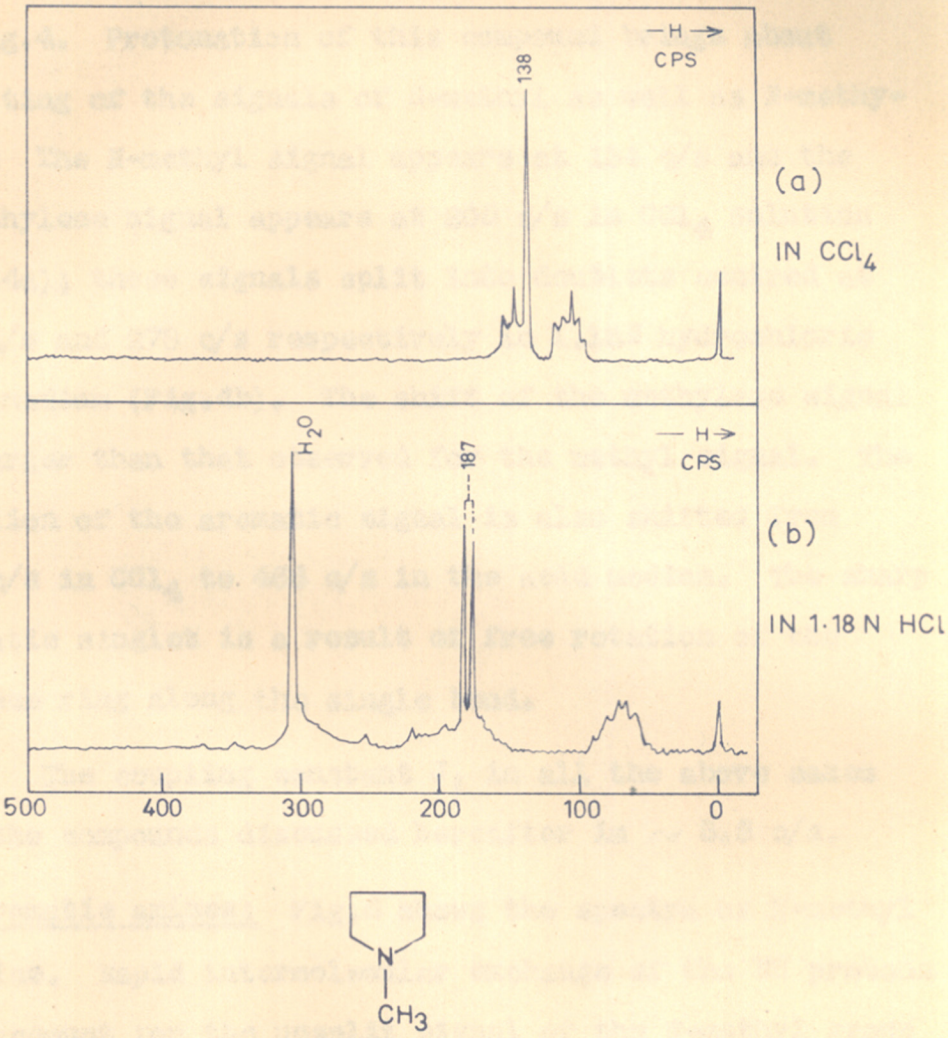


FIG. 3. NMR SPECTRA OF N-METHYL PYRROLIDINE

The spectra of N,N-dimethyl benzylamine are shown in Fig.4. Protonation of this compound brings about splitting of the signals of N-methyl as well as N-methylene. The N-methyl signal appears at 134 c/s and the N-methylene signal appears at 205 c/s in CCl_4 solution (Fig.4a); these signals split into doublets centred at 186 c/s and 273 c/s respectively in 1.18N hydrochloric acid medium (Fig.4b). The shift of the methylene signal is larger than that observed for the methyl signal. The position of the aromatic signal is also shifted from 434 c/s in CCl_4 to 466 c/s in the acid medium. The sharp aromatic singlet is a result of free rotation of the benzene ring along the single bond.

The coupling constant J, in all the above cases and the compounds discussed hereafter is $\sim$ 5.5 c/s.

2. Aromatic amines: Fig.5 shows the spectra of N-methyl aniline. Rapid intermolecular exchange of the NH protons may account for the unsplit signal of the N-methyl group in CCl_4 solution, which appears in the spectrum (Fig.5a) at 162 c/s. The broad signal at 201 c/s is assignable to the NH proton and the aromatic protons give rise to a multiplet in the region 377-440 c/s. The spectrum (Fig.5b) of the same compound in 1.18N hydrochloric acid shows the N-methyl signal shifted downfield to 204 c/s, as has

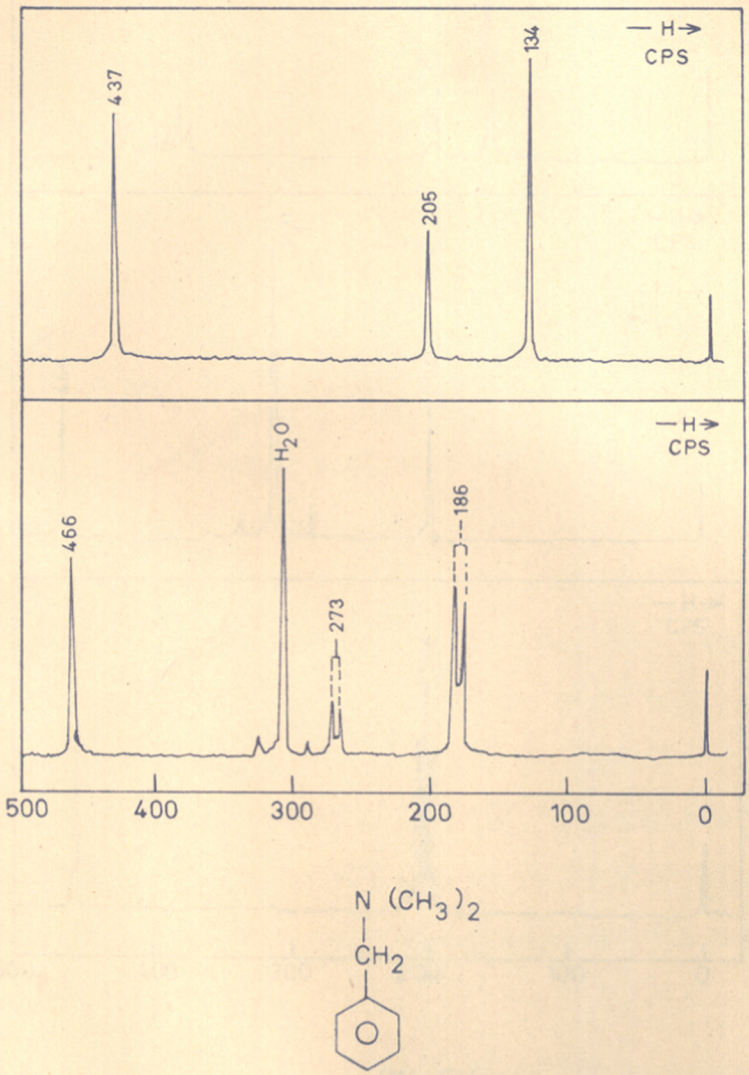


FIG. 4. NMR SPECTRA OF N N-DIMETHYL BENZYLAMINE

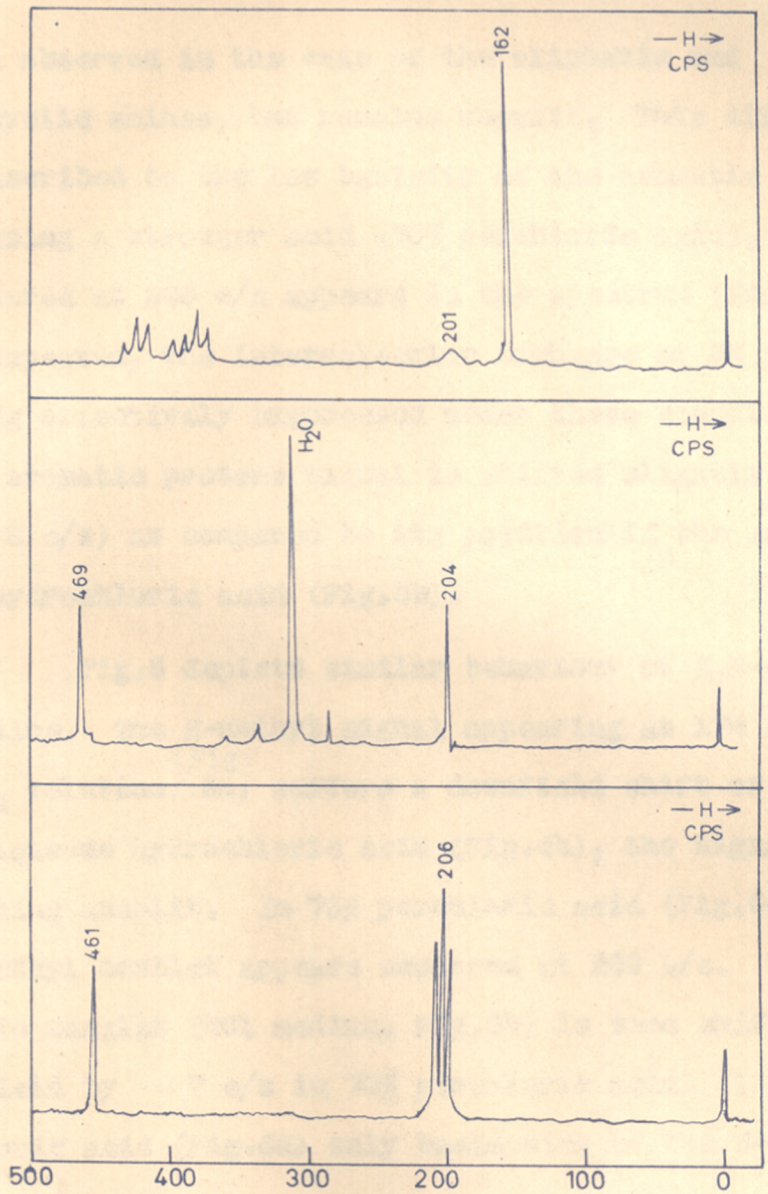


FIG. 5. NMR SPECTRA OF N-METHYL ANILINE

been observed in the case of the aliphatic and alicyclic amines, but remains unsplit. This difference is ascribed to the low basicity of the aromatic amine. By using a stronger acid (70% perchloric acid), a triplet centered at 206 c/s appears in the spectrum (Fig.5c) as expected, the intermolecular exchange of NH protons being effectively suppressed under these conditions. The aromatic protons signal is shifted slightly upfield ($\sim$8 c/s) as compared to its position in the spectrum in hydrochloric acid (Fig.5b).

Fig.6 depicts similar behaviour of N,N-dimethyl aniline. The N-methyl signal appearing at 174 c/s in CCl_4 solution (Fig. 6a) suffers a downfield shift of 37 c/s in aqueous hydrochloric acid (Fig.6b), the signal remaining unsplit. In 70% perchloric acid (Fig.6c) the N-methyl doublet appears centered at 209 c/s. The aromatic singlet (HCl medium, Fig.6b) is seen shifted upfield by $\sim$ 7 c/s in 70% perchloric acid. In 42% perchloric acid ~~(Fig.6d)~~ only broadening of the N-methyl signal occurs.

3. Cyclic imides: Fig.7 and 8 show the spectra of N-methyl pyrrolidone and theophylline respectively. Unlike the aromatic amines discussed earlier, these compounds do

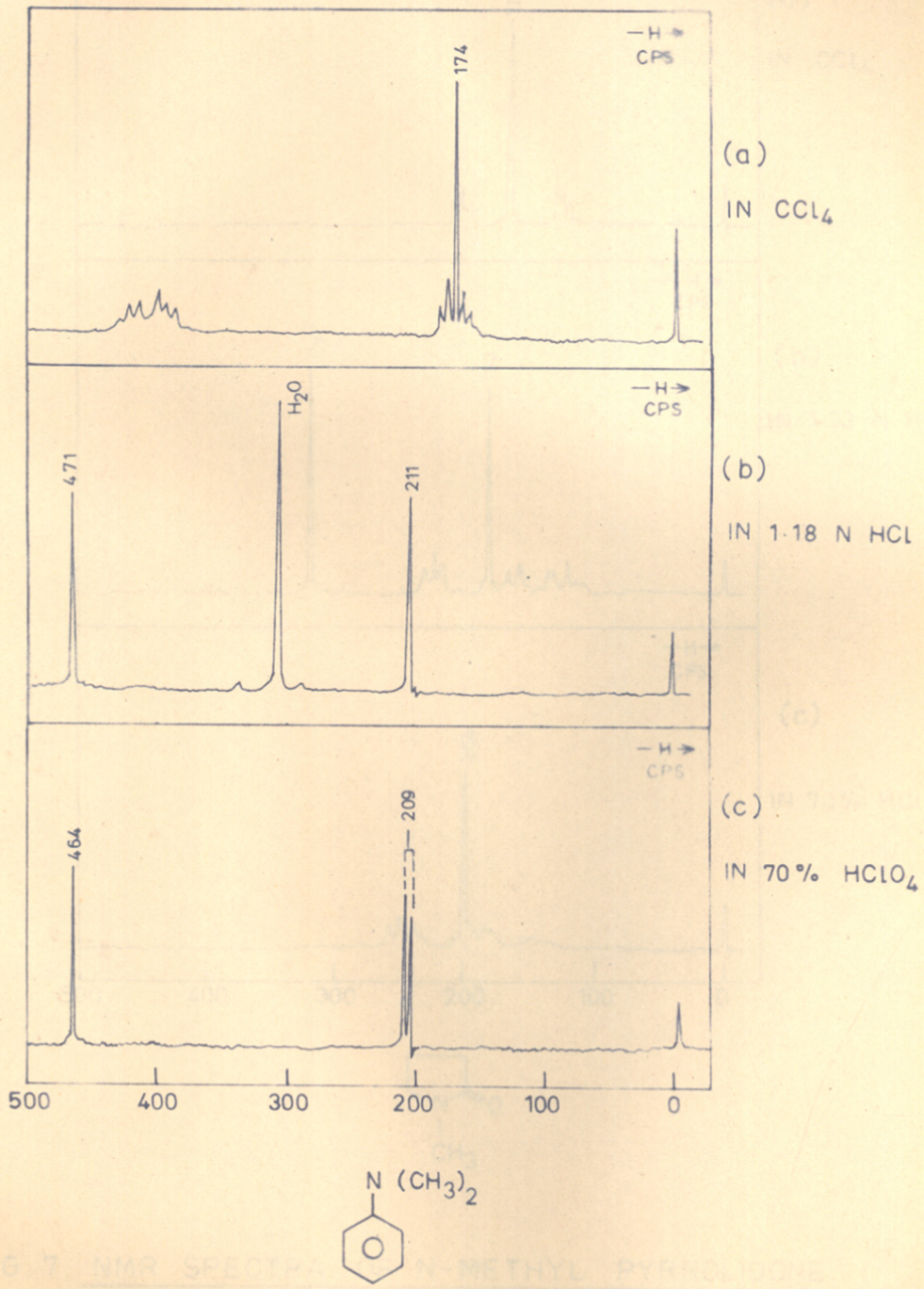


FIG. 6. NMR SPECTRA OF N, N-DIMETHYL ANILINE

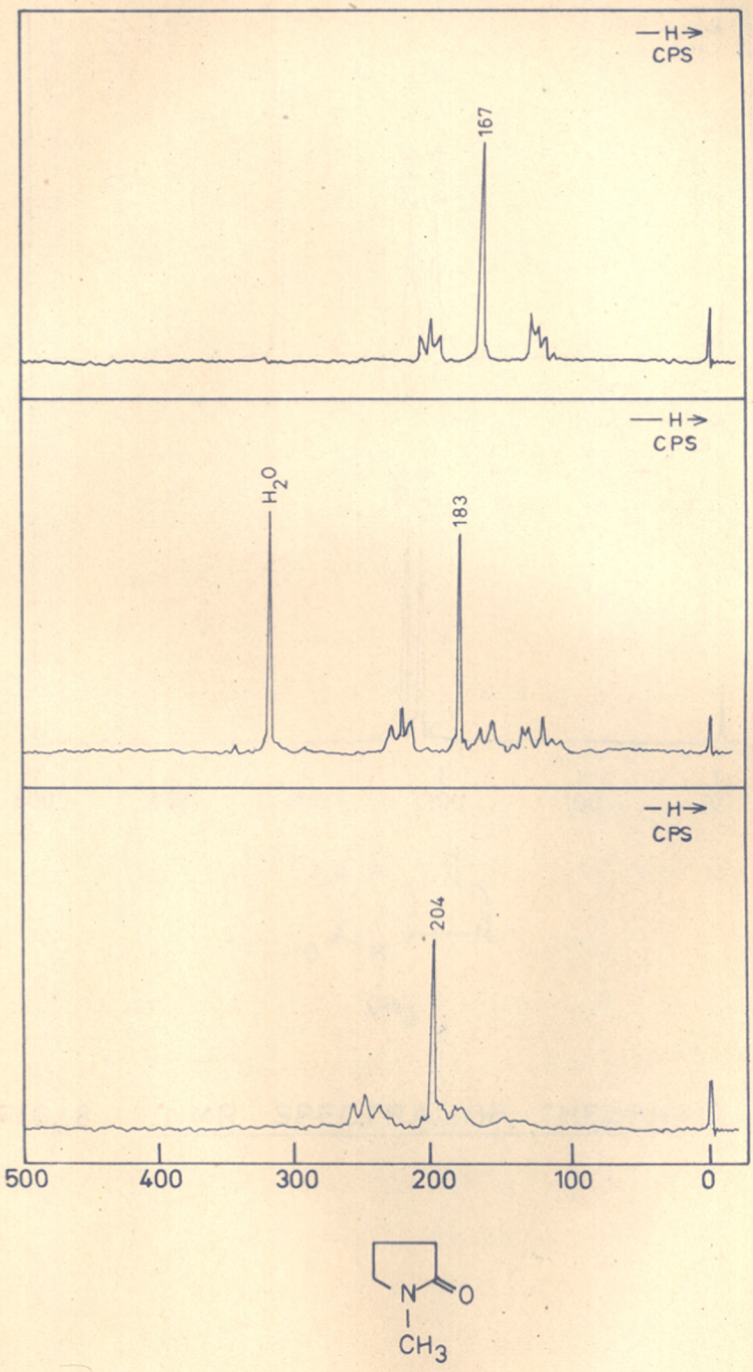


FIG. 7. NMR SPECTRA OF N-METHYL PYRROLIDONE

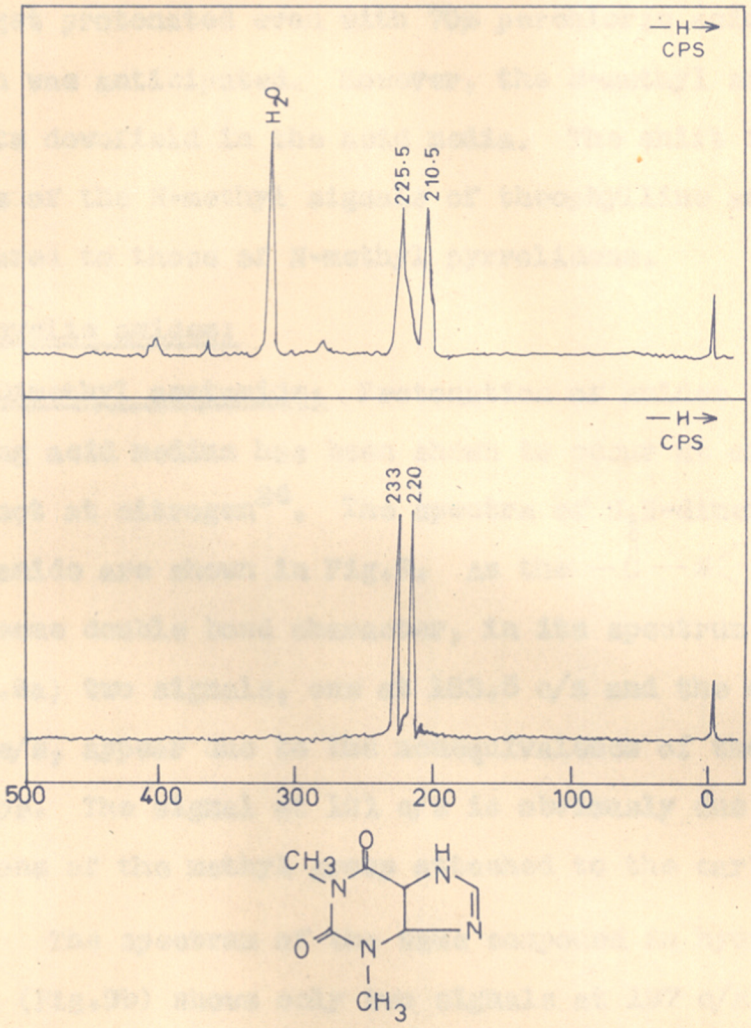


FIG. 8. NMR SPECTRA OF THEOPHYLLINE

not get protonated even with 70% perchloric acid, which was anticipated. However, the N-methyl signal shifts downfield in the acid media. The shift increments of the N-methyl signals of theophylline are small compared to those of N-methyl pyrrolidone.

4. Acyclic amides:

N,N-Dimethyl acetamide: Protonation of amides in strong acid medium has been shown to occur at oxygen and not at nitrogen[24]. The spectra of N,N-dimethyl acetamide are shown in Fig.9. As the $-\overset{\overset{O}{\|}}{C}-N<$ bond has some double bond character, in its spectrum in CCl_4 (Fig.9a) two signals, one at 183.5 c/s and the other at 174 c/s, appear due to the nonequivalence of the N-methyl groups. The signal at 121 c/s is obviously due to the protons of the methyl group attached to the carbonyl[24].

The spectrum of the same compound in hydrochloric acid (Fig.9b) shows only two signals at 197 c/s and 145 c/s having an integration ratio 2:1. The signal at 197 c/s assignable to the N-methyl groups indicates equivalence of the N-methyl groups. The structures of the O- and N-protonated species I and II respectively are given below.

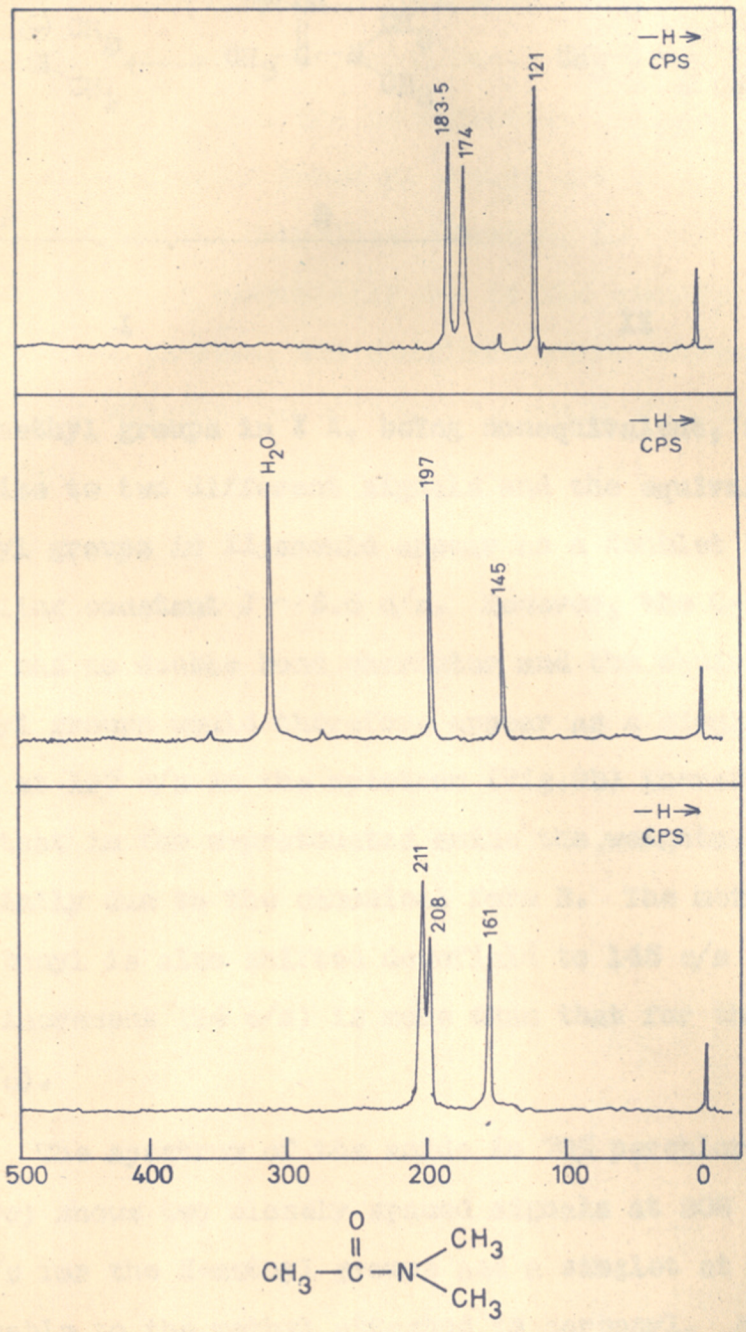
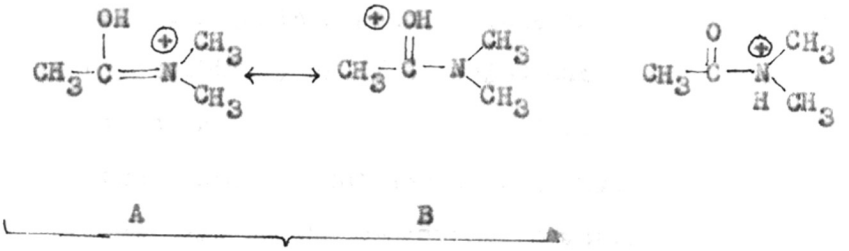


FIG. 9. NMR SPECTRA OF N,N-DIMETHYL ACETAMIDE



A B

I II

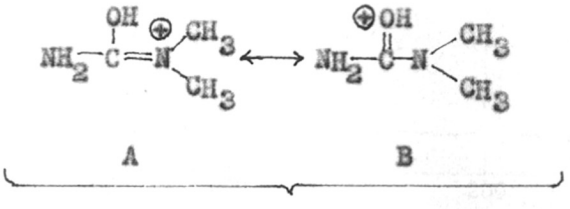
The N-methyl groups in I A, being nonequivalent, should give rise to two different signals and the equivalent N-methyl groups in II should appear as a doublet having a coupling constant $J \sim 5.5$ c/s. However, the C-N bond in I B has no double bond character and the equivalent N-methyl groups would therefore appear as a singlet. The signal at 197 c/s in the spectrum (Fig.9b) therefore indicates that in the O-protonated amide the weightage is essentially due to the canonical form B. The methyl attached to carbonyl is also shifted downfield to 145 c/s but its shift increment (24 c/s) is more than that for the N-methyl (18 c/s).

The spectrum of the amide in 70% perchloric acid (Fig.9c) shows two closely spaced signals at 208 c/s and 211 c/s for the N-methyl groups and a singlet at 161 c/s assignable to the methyl attached to carbonyl. As both the

N-methyl signals appear in less than 5.5 c/s width, the possibility of II is ruled out. The N-methyl signals are therefore due to different chemical shifts of two nonequivalent N-methyl groups and it is thus concluded that in the O-protonated amide, in this case, the weightage is essentially due to the canonical form I A. As can be seen, the downfield shift increment for the methyl attached to carbonyl is again more (16 c/s) than that for the N-methyl groups (12 c/s).

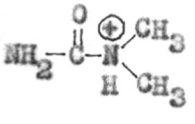
Asym. N,N-dimethyl urea

The spectra in Fig.10 show the behaviour of asym. N,N-dimethyl urea. The singlet appearing at 178 c/s in its pyridine solution (Fig.10a) moves downfield and appears at 192 c/s on protonation with hydrochloric acid (10b). Considering the structures III and IV for the protonated amide,



A B

III



IV

it is concluded on the arguments put forth for N,N-dimethyl acetamide, that under these conditions, weightage of the

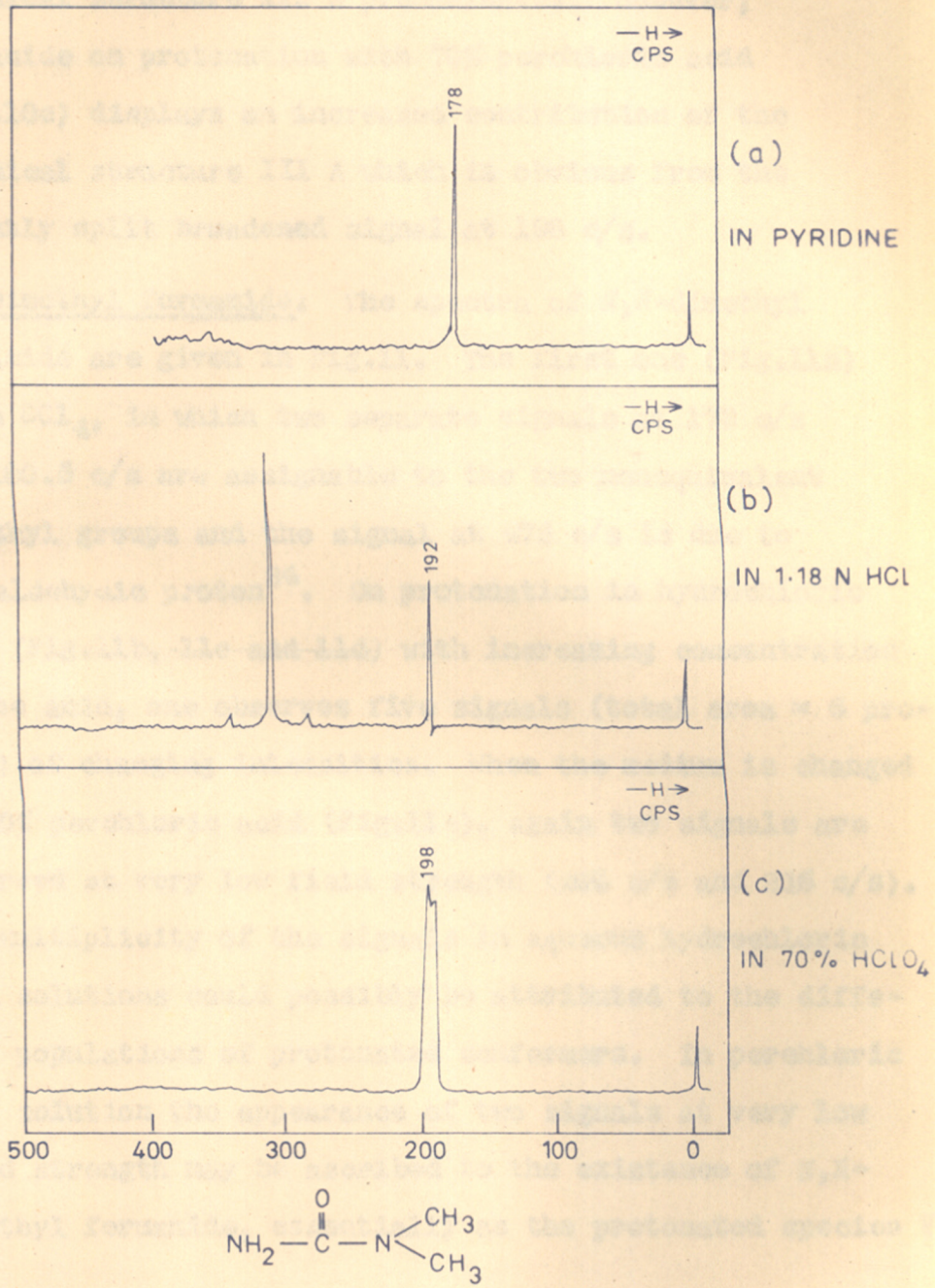
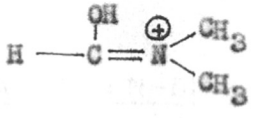


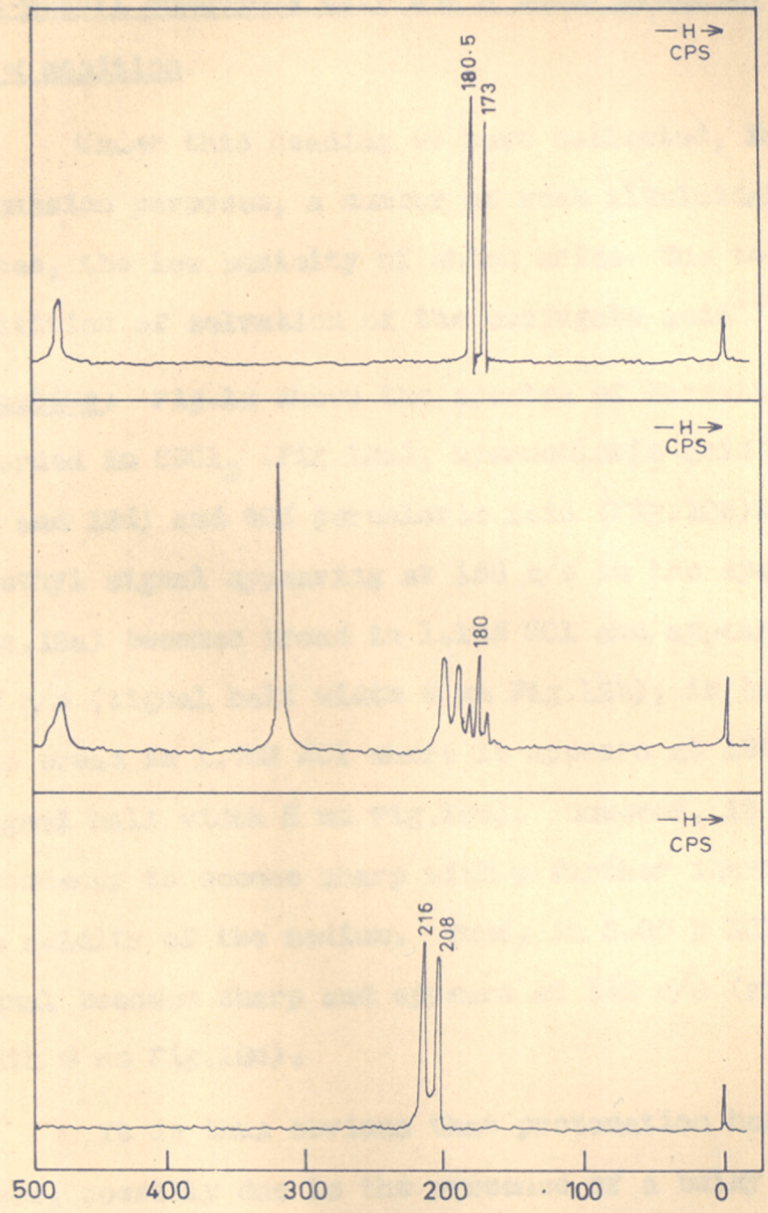
FIG. 10. NMR SPECTRA OF ASYM. N,N-DIMETHYL UREA.

canonical structure III B predominates. However, the amide on protonation with 70% perchloric acid (Fig.10c) displays an increased contribution of the canonical structure III A which is obvious from the slightly split broadened signal at 198 c/s.

N,N-Dimethyl formamide: The spectra of N,N-dimethyl formamide are given in Fig.11. The first one (Fig.11a) is in CCl_4, in which two separate signals at 173 c/s and 180.5 c/s are assignable to the two nonequivalent N-methyl groups and the signal at 476 c/s is due to the aldehydic proton[24]. On protonation in hydrochloric acid (Fig.11b, ~~11c and 11d~~) with increasing concentration of the acid, one observes five signals (total area = 6 protons) of changing intensities. When the medium is changed to 70% perchloric acid (Fig.11e), again two signals are observed at very low field strength (208 c/s and 216 c/s). The multiplicity of the signals in aqueous hydrochloric acid solutions could possibly be attributed to the different populations of protonated conformers. In perchloric acid solution the appearance of two signals at very low field strength may be ascribed to the existence of N,N-dimethyl formamide, essentially as the protonated species V



V



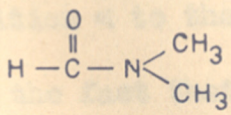


FIG.11. NMR SPECTRA OF N,N-DIMETHYL FORMAMIDE

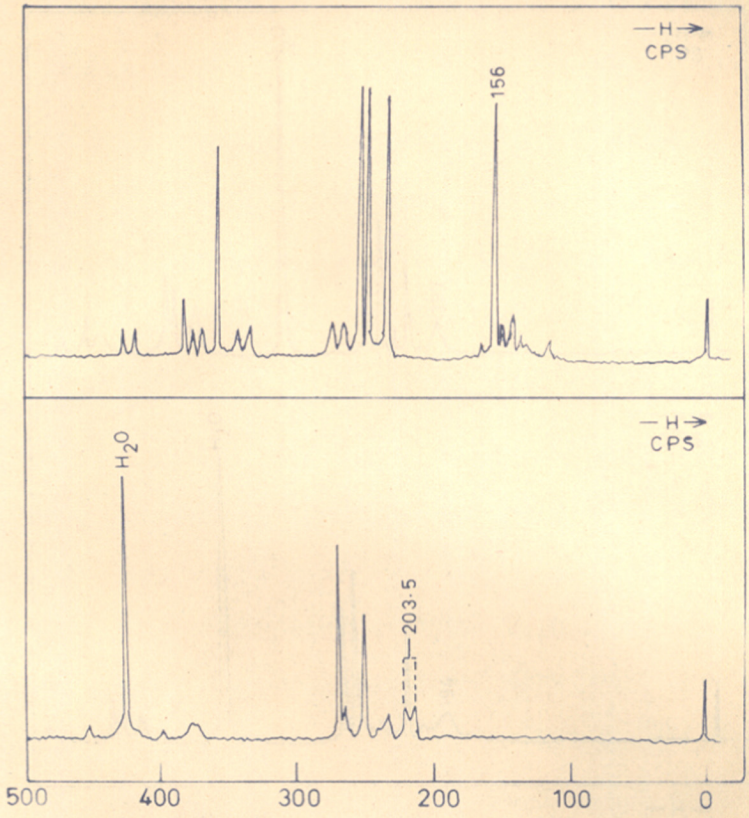
5. N-methyl compounds carrying a bulky group in the ∝ position.

Under this heading we have collected, for discussion purposes, a number of weak alkaloidal methylamines, the low basicity of which arise due to steric inhibition of solvation of the conjugate acid[25-27].

Narcotine: Fig.12 shows the spectra of narcotine recorded in $CDCl_3$ (Fig.12a), hydrochloric acid (Fig.12b, 12c and 12d) and 42% perchloric acid (Fig.12e). The N-methyl signal appearing at 156 c/s in the spectrum (Fig.12a) becomes broad in 1.18N HCl and appears at 197 c/s (signal half width 6 mm Fig.12b); it becomes more broad in 1.73N HCl where it appears at 194 c/s (signal half width 8 mm Fig.12c). However, it shows a tendency to become sharp with a further increase in the acidity of the medium. Thus, in 3.00 N HCl, the signal becomes sharp and appears at 192 c/s (signal half width 6 mm Fig.12d).

It is thus obvious that protonation has not been place, possibly due to the presence of a bulky group located in the position ∝ to the N-methyl group.

In view of the fact that the narcotine molecule does not have any exchangeable protons, the broadening



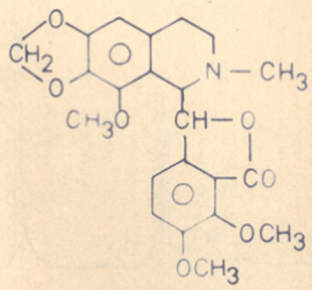


FIG. 12. NMR SPECTRA OF NARCOTINE

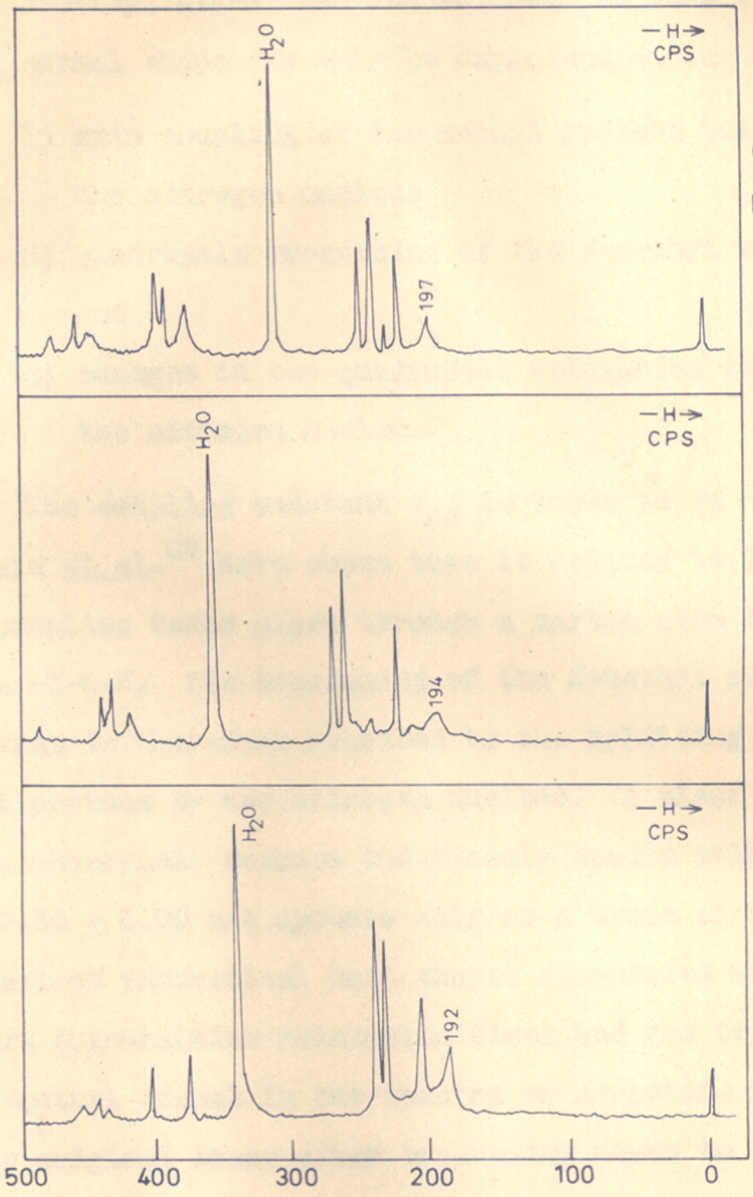


FIG. 12. NMR SPECTRA OF NARCOTINE.

of the N-methyl signal and its tendency to revert to its normal shape can only be explained on the basis of

1) spin coupling of the methyl protons with the nitrogen nucleus
2) quadrupole broadening of the N-methyl signal and
3) changes in the quadrupole relaxation time of the nitrogen nucleus.

The coupling constant J_{HN} is known to be ~ 50 c/s[28]. Grunwald et al.[29] have shown that it reduces to 0.54 ± 0.06 c/s when coupling takes place through a carbon atom in the system -N-C-H. The broadening of the N-methyl signal in narcotine is therefore ascribed to the splitting of the methyl protons by the nitrogen nucleus. A clear splitting is not observable because the closely spaced triplet with $J \sim 0.54 \pm 0.06$ c/s appears only as a broad signal. Pople[30] has derived theoretical band shapes associated with various spin-lattice relaxation times and the tendency of the N-methyl signal in the spectra of narcotine to revert to its original shape after broadening seems to be due to the rapid relaxation rate of the nitrogen nucleus i.e. decrease in its relaxation time.

The protonation could be effected with a stronger

acid (42% perchloric acid). Accordingly, the N-methyl signal in the spectrum (Fig.12e) has split into a doublet centered at 213.5 c/s.

The above results of narcotine may be compared with those of morphine, in which the absence of any bulky substituent in the position α to the N-methyl group leads to easy protonation. Accordingly, the N-methyl signal appearing at 145 c/s in pyridine (Fig.13a) splits into a doublet centered at 170 c/s in aqueous hydrochloric acid* (Fig.13b).

Nicotine: Another example in this group is that of nicotine, the spectra of which are given in Fig.14. The N-methyl signal appearing at 131 c/s in CCl_4 (Fig.14a) becomes broad in 1.18N hydrochloric acid (Fig.14b, signal half width** 5 mm) and appears at 193 c/s. The signal becomes sharp in more concentrated acid media (Fig.14c and 14d; signal half widths** 4.5 mm and 3 mm respectively), appearing at 193 c/s and 194 c/s respectively, and this is attributed to the change in the relaxation time of the nitrogen nucleus as explained earlier.

---

* ~ 0.12 molar solution in 3.00N hydrochloric acid had to be used for recording the spectrum due to poor solubility of the compound. Hence the downfield shift reported here has only a little significance.

** Signal half width measured for the N-methyl signals recorded at 250 c/s sweep width.

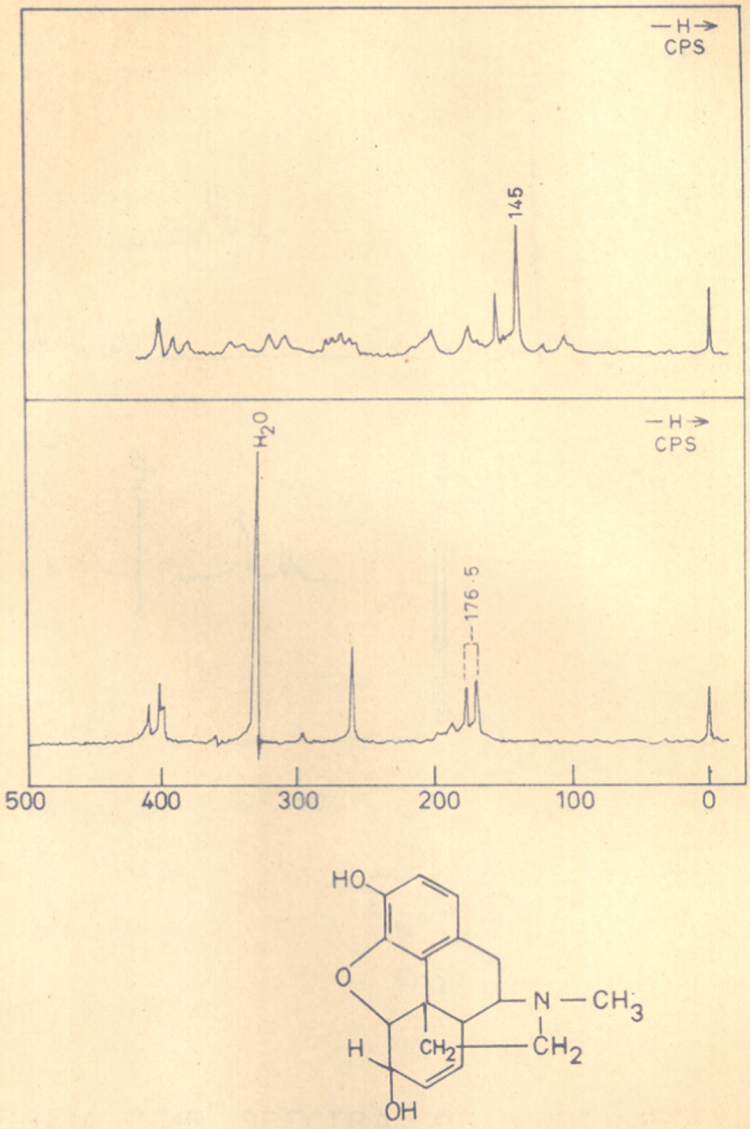


FIG. 13. NMR SPECTRA OF MORPHINE

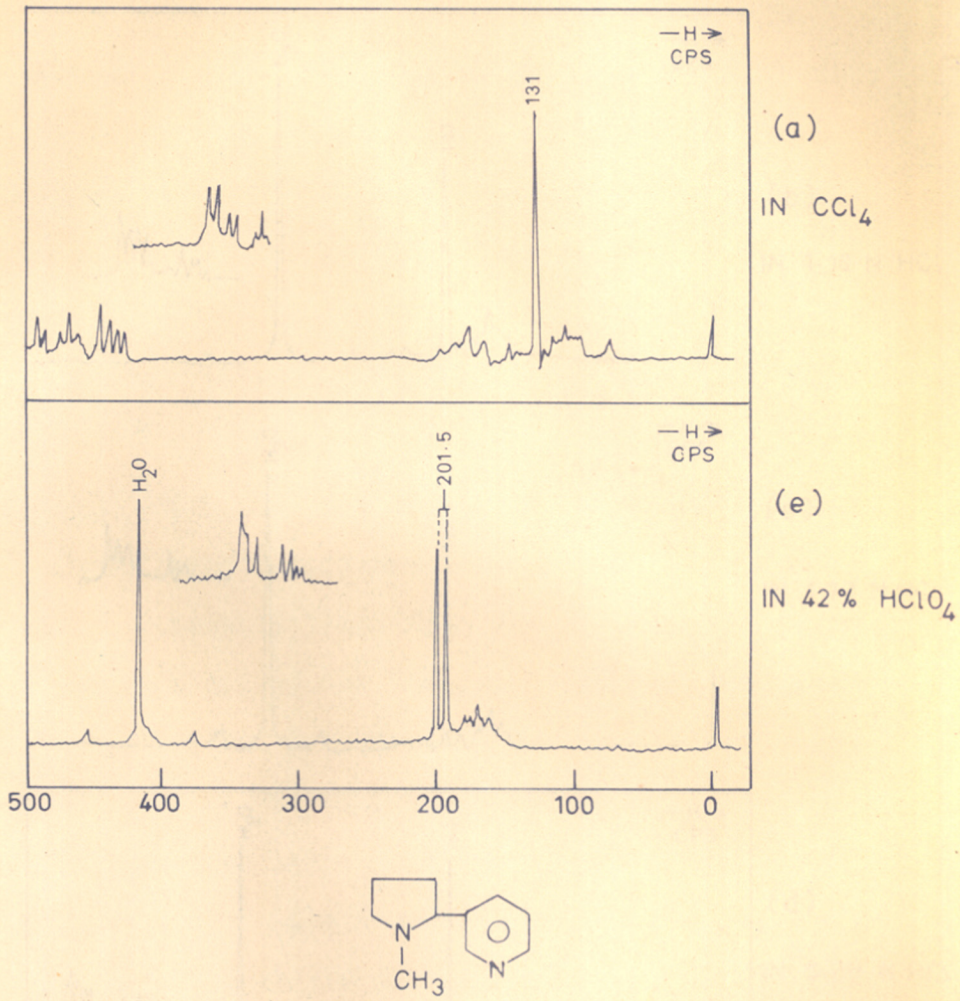


FIG. 14. NMR SPECTRA OF NICOTINE.

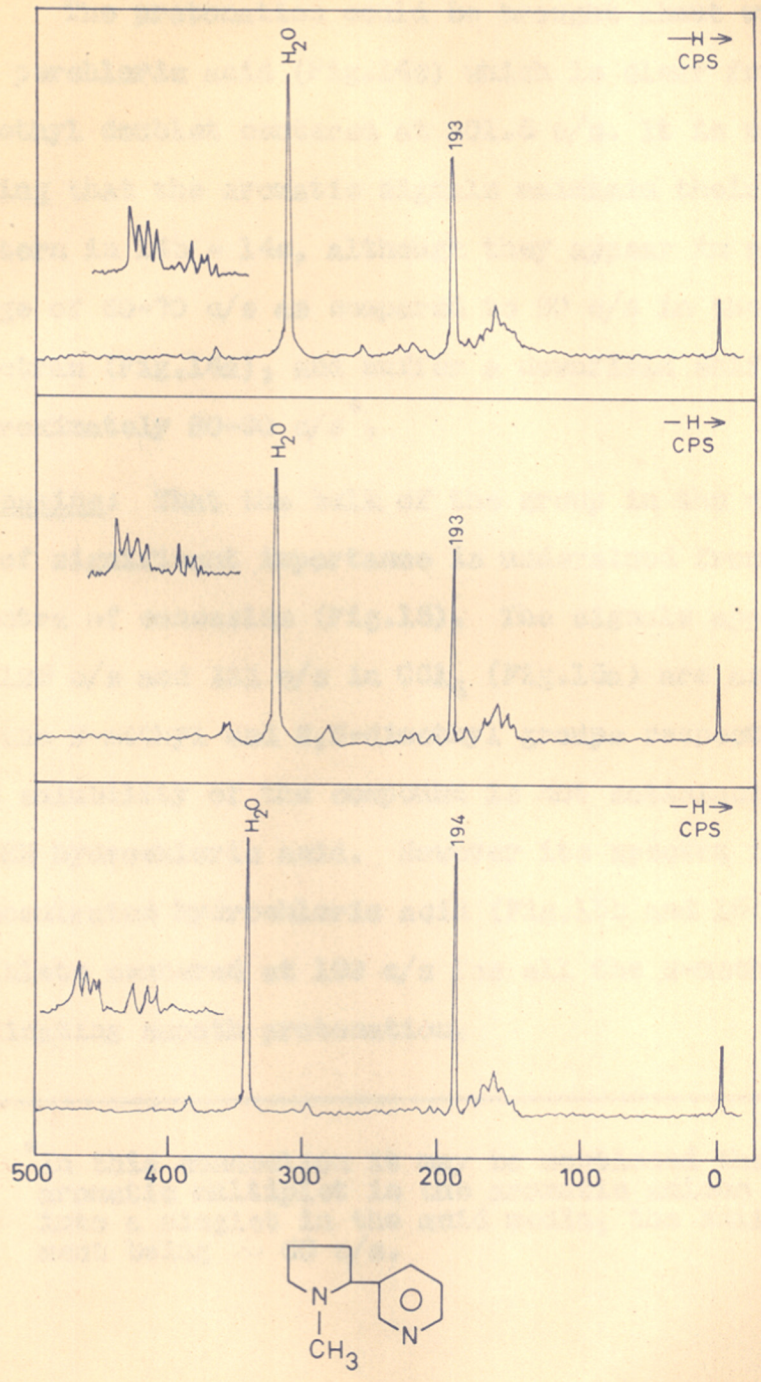


FIG.14. NMR SPECTRA OF NICOTINE.

The protonation could be brought about with 42% perchloric acid (Fig.14e) which is clear from the N-methyl doublet centered at 201.5 c/s. It is worth noting that the aromatic signals maintain their usual pattern in 14b - 14e, although they appear in a smaller range of 60-70 c/s as compared to 90 c/s in the original spectrum (Fig.14a), and suffer a downfield shift of approximately 80-90 c/s*.

Conessine: That the bulk of the group in the ∝ position is of significant importance is understood from the spectra of conessine (Fig.15). The signals appearing at 125 c/s and 131 c/s in CCl_4 (Fig.15a) are assignable to the N-methyl and N,N-dimethyl groups respectively. The solubility of the compound is not satisfactory in 1.18N hydrochloric acid. However its spectra in more concentrated hydrochloric acid (Fig.15b and 15c) show doublets centered at 199 c/s for all the N-methyl groups, indicating smooth protonation.

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*In this connection it may be mentioned that the aromatic multiplet in the aromatic amines collapsed into a singlet in the acid media, the shift increment being ~ 60 c/s.

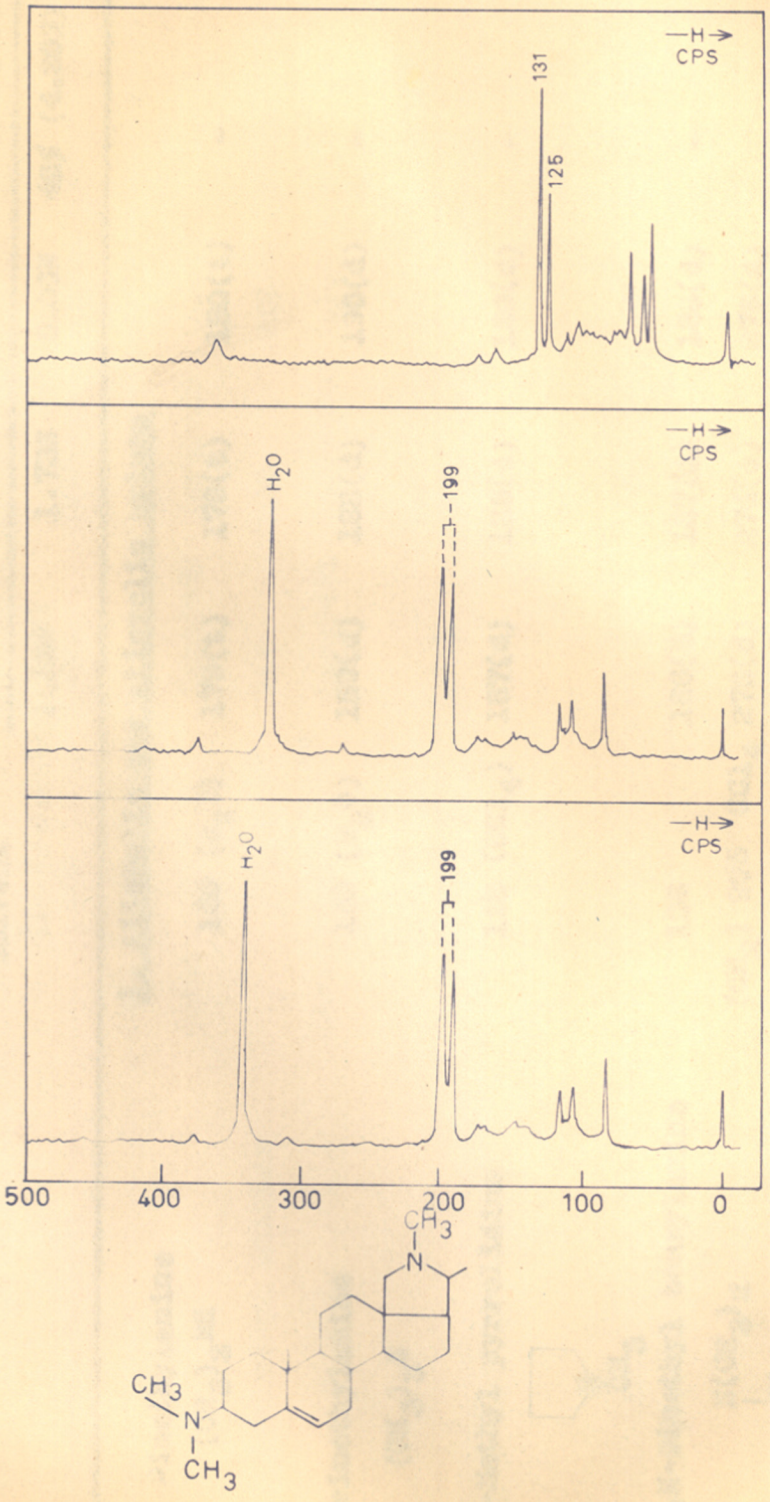
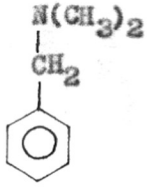
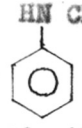
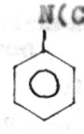
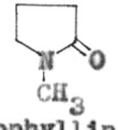
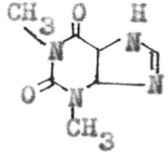
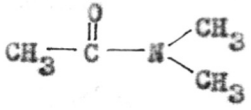
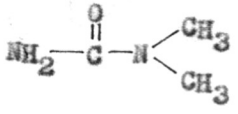
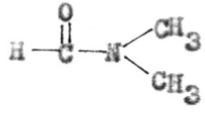


FIG. 15. NMR SPECTRA OF CONESSINE

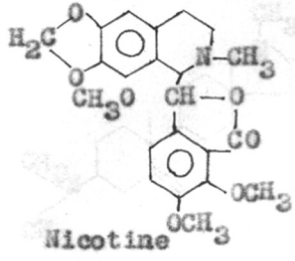
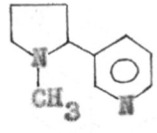
TABLE I - CHEMICAL SHIFT DATA OF N-METHYL GROUP IN DIFFERENT TYPES OF COMPOUNDS

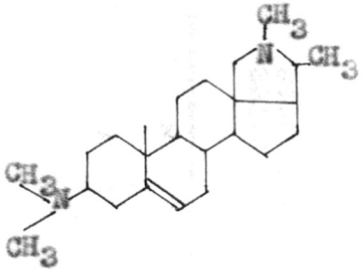
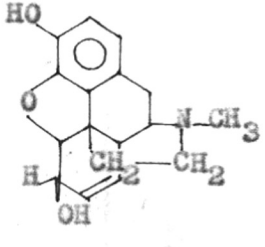
Compound	δ c/s in					
	Solvent	Hydrochloric acid			Perchloric acid	
		1.18N	1.73N	3.00N	42% (4.20N)	70% (7.00N)
I. Aliphatic and alicyclic amines						
Dimethylamine $(CH_3)_2NH$	150 (H_2O)	179(t)	179(t)	180(t)	-	-
Trimethylamine $(CH_3)_3N$	159 (H_2O)	189(d)	188(d)	190(d)	-	-
N-Methyl pyrrolidine 	138 (CCl_4)	187(d)	185(d)	189(d)	-	-
N,N-Dimethyl benzylamine 	134	186(d)	187(d)	188(d)	-	-
	(CH_2) 205 CCl_4	273(d)	274(d)	275(d)		
	(aro) 437	466	466	467		

Compound	δ c/s in					
	Solvent	Hydrochloric acid			Perchloric acid	
		1.18N	1.73N	3.00N	42% (4.20N)	70% (7.00N)
II. Aromatic amines						
N-Methyl aniline 	162	204	202	201	-	206(t)
	(NH) 201 CCl_4					
	(aro) 377-440	(aro)469	(aro)469	(aro)468		(aro)461
N,N-Dimethyl aniline 	174	211	210	212	212 (broad)	209(d)
	(aro) 388-438 CCl_4	(aro)471	(aro)470	(aro) 471	(aro) 467	(aro)464
III. Cyclic imides						
N-Methyl pyrrolidone 	167(CCl_4)	184	183	189	-	204
Theophylline 	209.4 (CDCl$_3$) - 219.6		210.5 225.5	-	-	220 233
	reported[21]					

Compound	δ c/s in					
	Solvent	Hydrochloric acid			Perchloric acid	
		1.18N	1.73N	3.00N	42% (4.20N)	70% (7.00N)
IV. Acyclic amides						
N,N-Dimethyl acetamide 	174 183.5 (CCl_4)	197	201	204	-	208 211
	($COCH_3$)121	($COCH_3$)145	($COCH_3$)151	($COCH_3$) 154		($COCH_3$) 161
Asymmetric N,N-dimethyl urea 	178 (Pyridine)	192	190	194	-	198
N,N-Dimethyl formamide 	173 180.5 (CCl_4)	181*	180*	182*	-	208 216
	(ald) 476	(ald) 495 512	(ald) 486 501	(ald) 492 504	-	-

*Indicates the position of the tallest signal in the group.

Compound	δ c/s in				
	Solvent	Hydrochloric acid			Perchloric acid	
		1.18N	1.73N	3.00N	42% (4.20N)	70% (7.00N)
V. N-methyl compounds carrying a bulky group in the α position						
Narcotine 	156($CDCl_3$)	197	194	192	213.5(d)	-
Nicotine 	131 (aro) 424-514 (CCl_4)	193 (aro) 511-575	193	193 (aro) 508-577	194 (aro) 510-578	201.5(d) (aro) 505.5-565.5	-

Compound	δ c/s in				
	Solvent	Hydrochloric acid			Perchloric acid	
		1.18N	1.73N	3.00N	42% (4.20N)	70% (7.00N)
VI. Other compounds						
Conessine 	125	190.5(d) (0.25 molar soln)	199(d)	199(d)	-	-
	131					
Morphine 	145 (pyridine)	-	-	176.5(d) (0.12 molar soln)	-	-

CONCLUSION

The results described above extend further the data obtained by Ma and Warnhoff[21] and it has been shown that valuable further information can be obtained in going to still stronger acids like perchloric acid. The data obtained by these authors in trifluoroacetic acid has been compared with our results in perchloric acid in Table II.

TABLE II - CHEMICAL SHIFTS OF N-METHYL GROUP IN WEAK AMINES IN TRIFLUOROACETIC ACID AND PERCHLORIC ACID

Compound	δ c/s in		
	T.F.A.	$HClO_4$ 42%	$HClO_4$ 70%
Nicotine	186.0 (unsplit)	201.5 (d)	-
N-Methyl aniline	190.0 (unsplit) 195.0 (partially resolved triplet at 27°).	-	206.0 (t)
N,N-Dimethyl aniline	207.5 (d)	-	209.0 (d)
N-Methyl-2-pyrrolidone.	185.5 (unsplit)	-	204.0 (unsplit)
Theophylline	218.5](unsplit) 231.0]	-	220.0](unsplit) 233.0]

REFERENCES

1 "Quantitative Organische Mikroanalyse" by H. Roth, pp.287, 1958 edition, Springer-Verlag, Wien.

2 J. Herzig and H. Meyer, Ber. 27, 319 (1894) Monatshefte für Chemie 18, 379 (1897).

3 A. Friedrich, Mikrochemie 7, 185 (1929); ibid. 7, 195 (1929); Z. physiol. Chem. 163, 141 (1928).

4 A.A. Sirotenko, Mikrochim Acta 1 (1955).

5 S. Edlbacher, Z. physiol. Chem. 101, 278 (1918); J. Chem. Soc. 114(2), 336 (1918).

6 K.H. Slotta and G. Haberland, Ber. 65B, 127 (1932).

7 Fr. Franzen and H. Pauli, Mikrochim. Acta 845 (1955).

8 F. Vieböck and C. Brecher, Ber. 63, 3207 (1930).

9 M. Furter, Helv. Chim. Acta 21, 1151 (1938).

10 R. Belcher, M.K. Bhatty and T.S. West, J. Chem. Soc. 2393 (1958).

11 E.P. White, Ind. Eng. Chem. Anal. Ed. 16, 207 (1944).

12 Fr. Franzen, W. Disse, and K. Rysell, Mikrochim. Acta 44 (1953).

13 Fr. Franzen, K. Rysell and H. Hack, Mikrochim. Acta 708 (1954).

14 Fr. Franzen, W. Hegemann and W. Disse, Mikrochemie Ver Mikrochim. Acta 39, 277 (1952).

15 Fr. Franzen, K. Rysell and H. Schall, Mikrochim Acta 712 (1954).

16 R. Kuhn and H. Roth, Ber. 67B, 1458 (1934).

17 P. Haas, Mikrochemie, 7, 69 (1929).

18 L.M. Brancone, Proceedings of the International Symposium on Microchemical Techniques - 1961 Edited by N.D. Cheronis (Microchemical Journal Symposium series, Vol. II, pp.616) Interscience Publishers, New York.

19 H. Conroy, P.R. Brook, M.K. Rout and N. Silverman, J. Am. Chem. Soc. 80, 5178 (1958); also see: H. Gysel, Mikrochim. Acta, 743 (1954).

20 "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry" by L.M. Jackman, 1959 Edition, pp.56, Pergamon Press Ltd. London.

21 J.C.N. Ma and E.W. Warnhoff, Can. J. Chem. 43(6), 1849 (1965).

22 E. Grunwald, A. Lowenstein and S. Meiboom, J.Chem. Phys. 27, 630 (1957).

23 H.T. Clarke, H.B. Gillespie and S.Z. Weisshaus, J. Am. Chem. Soc. 55, 4571 (1933).

24 G. Fraenkel and C. Niemann, Proc. Nat. Acad. Sci. USA 44, 688 (1958).

25 J. Clark and D.D. Perrin, Quarterly Reviews XVIII(3), 295 (1964).

26 J.F. King in K.W. Bentley, editor, Elucidation of Structures by Physical and Chemical Methods, Part I in Technique of Organic Chemistry Vol.XI editor, A. Weissberger, Interscience Publishers, New York, 1963, Chapter 6.

27 H.C. Brown and B. Kanner, J. Am. Chem. Soc. 75, 3865(1953)

28 "Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry" by L.M. Jackman, 1959 edition, pp.72, Pergamon Press Ltd., London.

29 E. Grunwald, A. Lowenstein and S. Meiboom, J. Chem. Phys. 27, 641 (1957).

30 J.A. Pople, Molecular Phys. 1, 168 (1958).

SYNOPSIS

TITLE: "ANALYTICAL METHODS IN ORGANIC CHEMISTRY"

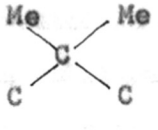
The thesis is presented in two parts. The first part relates to the chemical methods of group determination and consists of two chapters. The second part deals with the spectral methods of analysis and has been subdivided into three chapters.

PART I

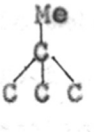
FUNCTIONAL GROUP ANALYSIS BY CHEMICAL METHODS

Chapter I - Differential Kuhn-Roth C-methyl estimation

The method described in this Chapter is a modification of the Kuhn-Roth oxidation and yields useful information about the number and nature of quaternary methyls in terpenoids and steroids. Although NMR spectroscopy can readily furnish this type of information, the present method gives useful data which cannot be obtained otherwise; e.g. the differential Kuhn-Roth estimation method has been applied in deciding whether a given compound contains grouping (A) or has two groupings of the type (B).



(A)



(B)

Chapter II - Determination of the number and nature of acetyl groups in organic compounds.

The existing chemical and physical methods do not seem to be quite adequate for deciding the nature of the acetyl groups. As certain natural products are known to be acetylated compounds, this method would be helpful in yielding important information about the structure of such molecules and also for those natural products where acetylation of the active hydrogen atoms offers no difficulty.

The method is based on the different rates of hydrolysis of various types of compounds; it has been possible to distinguish between the acetyl groups attached to an oxygen atom and a nitrogen atom having different structural environments.

PART II

SOME APPLICATIONS OF SPECTROSCOPIC METHODS OF ANALYSIS

Chapter I - Scissoring frequency of methylene group flanking a carbonyl

The methylene groups adjacent to carbonyl are known to absorb in the region 1410-1430 cm^{-1} of the infrared spectrum. The shift of this band and its absolute intensity have been studied in a variety of cyclic ketones and useful correlations obtained regarding the effect of the ring size and other structural features on the position and the intensity of the band.

Chapter II - Identification of Methylnaphthalenes by NMR

This Chapter gives the details of an analytical method for a quick identification of an alkyl naphthalene, isolated as a trinitrobenzene complex. Alkyl naphthalenes constitute important dehydrogenation products of terpenoids. With the advent of gas-liquid chromatography it is possible to isolate almost all the dehydrogenation products from a given precursor. However, the products are very often obtained in small quantities and are purified and characterized as their complex with trinitrobenzene. It is demonstrated now that the PMR spectra of these complexes can directly furnish information which can enable one to characterize the alkyl naphthalene by the application of recently published PMR rules (B.A. Nagasampagi, R.C.Pandey, V.S. Pansare, J.R. Prahlad and Sukh Dev, Tetrahedron letters No.8, pp.411-416, 1964) for the identification of methyl naphthalenes.

Chapter III - Characterization of the N-methyl group in an organic compound by NMR

The usual chemical method for the determination of the N-methyl group has many drawbacks. This Chapter describes a nuclear magnetic resonance method developed for the characterization of this important functional group.

STATEMENT I

A critical survey of relevant literature available is presented in collated and condensed form in each part of the thesis. These portions in addition to being of interest in their own right, will provide the necessary background for the appraisal of the work described. The work presented is original and represents discovery of new facts and consists of bona fide record of the experimental work carried out by the candidate. Detailed information about the new facts observed and how they have advanced the knowledge of the subject have been fully dealt with in the synopsis.

STATEMENT II

The candidate has, with the concurrence of the University Teacher worked on "ANALYTICAL METHODS IN ORGANIC CHEMISTRY" suggested by Dr.Sukh Dev .

In keeping with the general practice in the reporting of scientific observations, due acknowledgement has been made wherever the work described is based on the findings of other investigators. A complete bibliography of the books and journals consulted is given at the end of each part.

B. Pansare

Signature of the Candidate.

Sukh Dev

Signature of the Guide.

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V. S. Pansare

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NCL, Poona 8

10th June 1966.