[CONTRIBUTION FROM THE INFRARED SPECTROSCOPY LABORATORY, FISK UNIVERSITY]

Infrared Spectroscopy of Compounds having Biological Interest. I. A Comparative Study of Normal and *i*-Steroids[1]

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The infrared spectra from 3700 cm.$^{-1}$ to 830 cm.$^{-1}$ (2.5 μ to 12.0 μ) have been reported for six pairs of *i*- and normal steroid isomers. Each of the twelve spectra is unique and can be used for purposes of identification. Several interesting band position shifts in going from solid to solution have been noted. One is able to distinguish between these two groups of steroids by a study of the entire spectrum, the *i*-steroids having, in general, a more pronounced and complex spectra and, in particular, a unique band *ca.* 890 cm.$^{-1}$. The possibility of assigning this band to the asymmetric deformation of the cyclopropane ring is discussed.

Introduction

It was announced in 1948[2] that an *i*-steroid compound, *i*-androstenolone, has been isolated in considerable amounts from the urine of human patients suffering from cancer of the adrenal gland. Previous to this date *i*-steroids have only been produced by *in vitro* synthesis.[3] It appeared valuable to add an infrared study of *i*-steroids to the present literature on the infrared spectroscopic study of steroids.[4,5,6,7] In two previous notes from this Laboratory[8,9] studies have been reported of the influence upon the CH and C=O stretching vibrations of the cyclopropane ring formed by the bridge bond extending from C_3 to C_5 in ring A. The present paper will give a more extensive comparative study of six pairs of normal and *i*-steroids in the region from 3700 cm.$^{-1}$ to 830 cm.$^{-1}$ (2.5 μ to 12 μ).

Apparatus, Calibration and Accuracy

The Fisk University Infrared spectrometer[10] used in this research is an automatic recording, single beam instrument built by the University of Michigan Physics Department Shop. Its optics differ from the original design,[11,12] being of the one-mirror Littrow-type mounting.[13] The resolution, however, is essentially the same. A breaker-type amplifier[14] is used to amplify the thermopile output signal in order that it may be recorded on a commercial potentiometer pen recorder.

In order to compensate partially for the emissivity characteristics of the source, the entrance and exit slits are gear driven to open in synchronism with the motor drive which rotates the prism table. Water vapor and carbon dioxide were partly removed from the optical path by trays of phosphorus pentoxide and sodium hydroxide. Rapid and more complete drying was obtained through continuous agitation of the air within the enclosed spectrometer and source. This was accomplished through the simple device of operating a tiny radio tube cooling fan within each case.[15]

The spectrometer was calibrated[16] by using known vibration-rotation bands of water vapor, carbon dioxide, methane and ammonia. The spectrometer is equipped with sodium chloride, lithium fluoride and potassium bromide prisms for use in studying different portions of the spectrum to the best advantage. Under the routine conditions set up for surveying spectra of samples rapidly with the rock salt prism, the positions of individual sharp bands are measurable and reproducible to an accuracy of ±10 cm.$^{-1}$ at 3300 cm.$^{-1}$, ±3 cm.$^{-1}$ at 1700 cm.$^{-1}$ and ±1 cm.$^{-1}$ at 1000 cm.$^{-1}$ (approximately 0.01 μ over the whole range) when the background interference is not too severe.

Throughout this paper the intensity symbols have the following significance: vw = very weak, w = weak, Mw = medium weak, M = medium, MS = medium strong, S = strong, and VS = very strong. The strongest band in any particular spectrum is termed S (or VS if 100% absorbing) and intensity symbols are thereafter assigned to all other bands relative to the strongest.

Experimental Data

Figure 1 is a set of line diagrams, showing frequencies and relative intensities in the crystalline state, of the twelve compounds studied.[17] Figure 2, a percentage transmission plot of the spectra for a pair of representative compounds, is included to illustrate the general appearance of the spectra obtained. The sodium chloride prism was used for all the work reported in this paper.

The compounds were prepared for analysis in the solid crystalline state (Nujol mull technique, the mass ratio of oil to solid being about 1:1) and in solution (about 30 mg./cc.) in carbon disulfide as well as in hexachlorobutadiene (cell thickness, 0.15 mm.). In the spectral regions where neither solvent had absorptions of its own, the two different solutions gave essentially identical spectral profiles for a given solute. The solution spectra were essentially the same as the solid spectra for a given compound with certain exceptions which will be discussed later.

Analysis of Spectra, 3700–1250 Cm.$^{-1}$

In this section absorption band assignments to hydrogen bond and double bond vibrations will be discussed.

OH Stretching Vibration.[4,5]—Only one pair of steroids studied, the methoxyandrosten-17β-ols, contained the hydroxyl group. The position of the band associated with the OH linkage is at 3425 cm.$^{-1}$ and 3460 cm.$^{-1}$ for the crystalline *n*- and *i*-steroid, respectively. In solution in both cases the bands shifted to 3625 cm.$^{-1}$, the usual position of the band of unassociated OH.

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(1) Presented in part before the Division of Physical and Inorganic Chemistry at the 119th Meeting of the American Chemical Society, Boston, Mass., April, 1951.

(2) E. Dingemanse, L. G. Huis in't Veld and S. L. Hartogh-Katz, *Nature*, **161**, 848 (1948); **162**, 492 (1948); D. H. R. Barton and W. Klyne, *ibid.*, **162**, 493 (1948).

(3) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd edition, Reinhold Publishing Corp., New York, N. Y., 1949, p. 256.

(4) R. F. Furchgott, H. Rosenkrantz and E. Shorr, *J. Biol. Chem.*, **163**, 375 (1946); **164**, 621 (1946); **167**, 627 (1947); **171**, 523 (1947).

(5) R. N. Jones, V. Z. Williams, M. J. Whalen and K. Dobriner, THIS JOURNAL, **70**, 2024 (1948).

(6) R. N. Jones, P. Humphries and K. Dobriner, *ibid.*, **71**, 241 (1949); **72**, 956 (1950).

(7) R. N. Jones, P. Humphries, E. Packard and K. Dobriner, *ibid.*, **72**, 86 (1950).

(8) M. L. Josien, *Compt. rend.*, **231**, 131 (1950).

(9) M. L. Josien and N. Fuson, *ibid.*, **231**, 1511 (1950).

(10) The Fisk University spectrometer is similar in most respects to the instrument described by E. A. Boettner in "Research Report No. 520," Wyandotte Chemicals Corporation, Wyandotte, Michigan, May 27, 1946.

(11) H. M. Randall and J. Strong, *Rev. Sci. Instr.*, **2**, 585 (1931).

(12) H. M. Randall, R. G. Fowler, N. Fuson and R. Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand and Co., Inc., New York, N. Y., 1949, p. 85 (discusses later developments of the original instrument).

(13) N. Wright, *Ind. Eng. Chem., Anal. Ed.*, **13**, 1 (1941).

(14) M. Liston, *Rev. Sci. Instr.*, **17**, 194 (1946).

(15) P. M. Tolliver, Thesis (unpublished) Fisk University, Nashville, Tennessee, 1951, p. 25.

(16) R. A. Oetjen, C. L. Kao and H. M. Randall, *Rev. Sci. Instr.*, **13**, 515 (1942).

(17) Numerical tables of wave numbers and relative intensities can be obtained by writing directly to the authors.

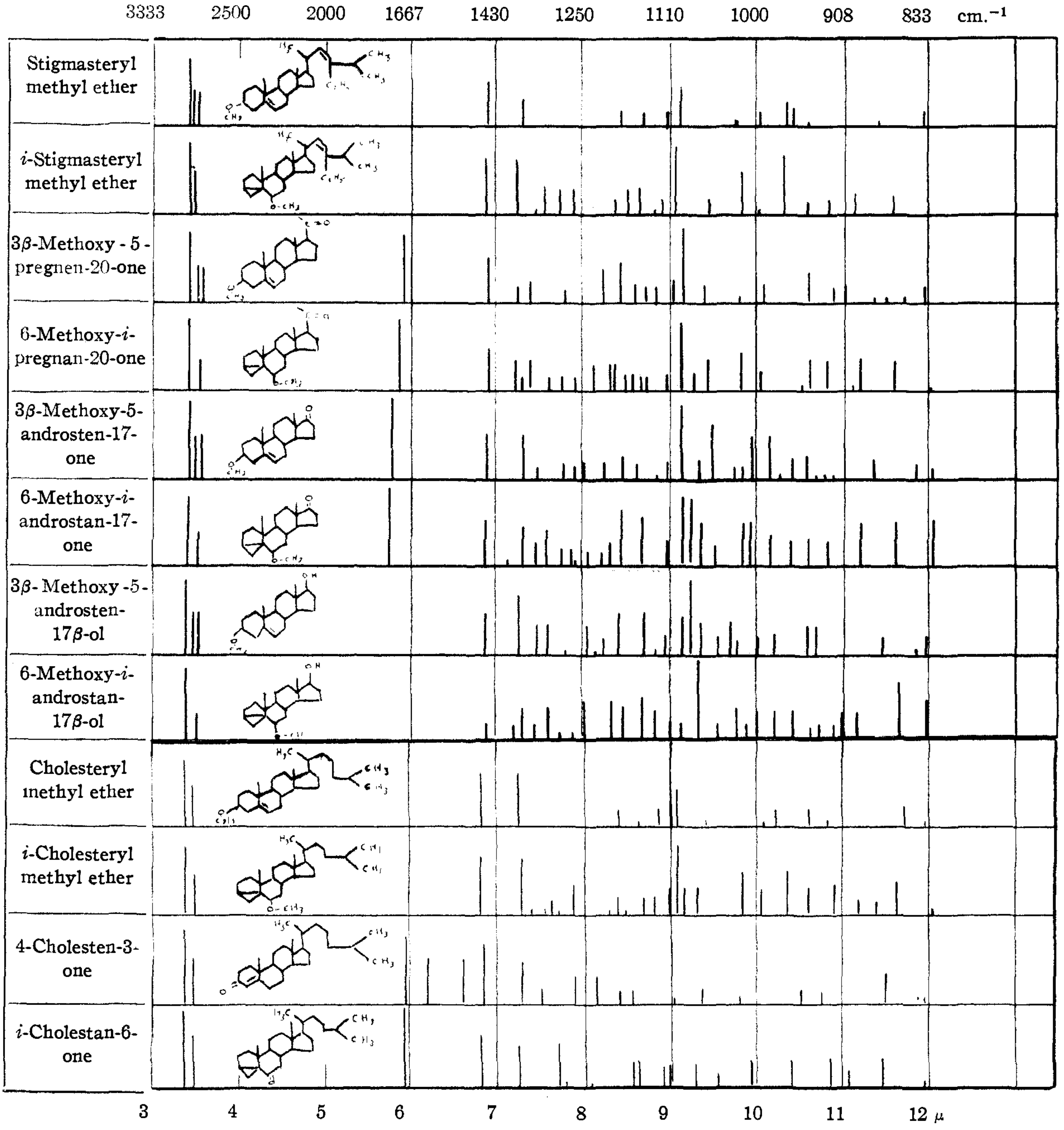

Fig. 1.—Line diagram of the infrared spectra of the normal and *i*-steroids.

CH Stretching Vibrations.—A point of inflection at 3030 cm.$^{-1}$ has generally been considered to be characteristic of the stretching vibration of the C–H group close to a double bond. It has been shown[8] that *i*-steroid spectra also have this point of inflection. With the rock salt prism used in the present work only an unresolved multiple band at *ca.* 2940 cm.$^{-1}$ was observed for both normal and *i*-steroids.

C=C Stretching Vibrations.—With the exception of 4-cholesten-3-one,[9] in which a medium band at 1610 cm.$^{-1}$ is assigned to the C=C conjugated with a carbonyl group, none of the steroids studied showed evidence of a band in the 6 μ region which could be assigned to the C=C group. This result is in agreement with Jones,[5] who states that the non-conjugated C=C cannot always be found because of its low intensity and the interference of the atmospheric water vapor spectrum.

C=O Stretching Vibrations.—Table I gives the comparative results for the absorption bands associated with the carbonyl groups, for both solid and solution samples. The position of these bands is found to be influenced by the environment of the carbonyl group. It should be noted that the shift of band position in going from crystalline solid to solution is very small (3 cm.$^{-1}$ to 6 cm.$^{-1}$) in all cases except for that of *i*-cholestan-6-one in which the shift is 12 cm.$^{-1}$ to higher frequencies. In this particular case, therefore, a study of the carbonyl band shift in going from solid to solution would enable the two isomers to be distinguished.

CH Bending Vibrations.—As is to be expected, all twelve steroids have medium intense bands at

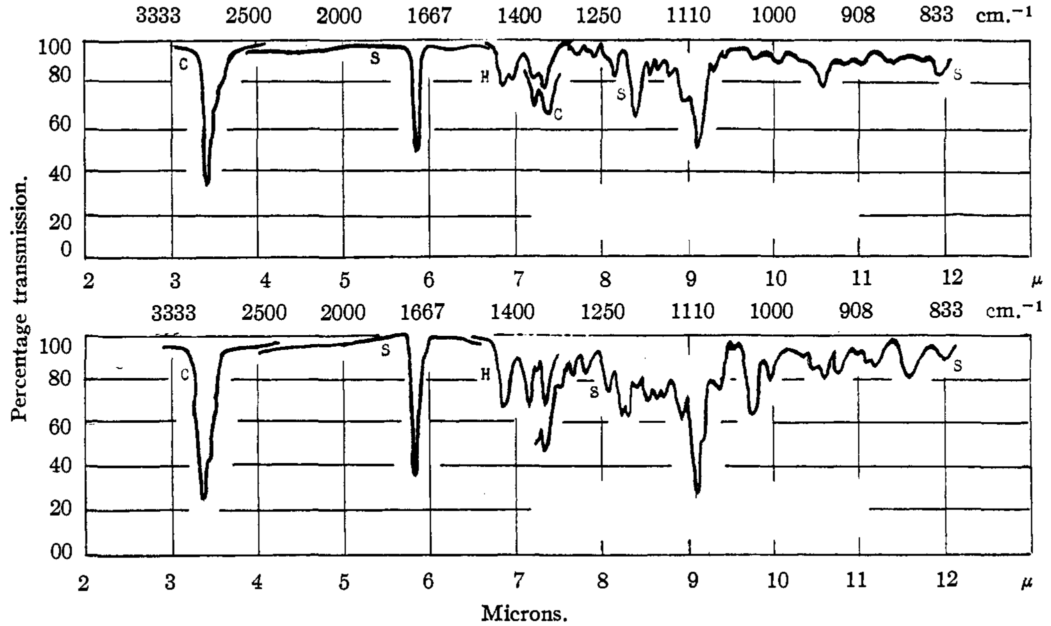

Fig. 2.—Per cent. transmission curves for 3β-methoxy-5-pregnen-20-one (above) and 6-methoxy-*i*-pregnan-20-one (below). The curves shown are those for the sample in solid form in paraffin oil paste (S) except in the 3000 cm.$^{-1}$ and 1400 cm.$^{-1}$ regions in which absorptions produced by the paraffin oil mask those of the samples. In these two regions solution spectra in carbon disulfide (C) and hexachlorobutadiene (H) have been given.

TABLE I

Compound	Position of C=O group (environment effect)	Frequency in cm.$^{-1}$ Solid (oil paste)	Solution (in CS_2 or C_4Cl_6)
3β-Methoxy-5-pregnen-20-one	Side chain	1704	1709
6-Methoxy-*i*-pregnan-20-one	(α-Position)	1712	1709
3β-Methoxy-5-androsten-17-one	5-Carbon ring D	1742	1748
6-Methoxy-*i*-androstan-17-one		1742	1748
4-Cholesten-3-one	6-Carbon ring A	1678[a]	1680[a,b]
i-Cholestan-6-one	6-Carbon ring B	1683[a]	1695[a,c,d]

[a] M.-L. Josien and N. Fuson, *Compt. rend.*, **231**, 1511 (1950). [b] R. N. Jones, *et al.*, THIS JOURNAL, **70**, 2024 (1948), give 1677 cm.$^{-1}$ for 4-cholesten-3-one in carbon disulfide solution. [c] A result very similar to this for *i*-cholestan-6-one in carbon tetrachloride solution has been found independently by R. Norman Jones (private communication, Dec. 27, 1950). [d] While this article was in press, we obtained, through the courtesy of Dr. D. H. R. Barton, Birkbeck College Research Laboratory, University of London, a sample of *i*-androstan-6,17-dione. Its infrared spectrum reinforced the significance of the results in this table, its two carbonyl bands appearing at 1680 cm.$^{-1}$ and 1736 cm.$^{-1}$ in the solid state and at 1689 cm.$^{-1}$ and 1745 cm.$^{-1}$ in CS_2 solution.

about 1460 cm.$^{-1}$ and 1380 cm.$^{-1}$, these being characteristic of CH_2 and CH_3 bending vibrations.

Analysis of Spectra, 1250–830 Cm.$^{-1}$

The most obvious difference between *i*- and *n*-steroids in the low frequency region of the spectrum, as may be seen from Fig. 2, is the presence of more and stronger lines in the *i*-steroids spectra. The following paragraphs will contain a discussion of the bands which persist to a significant extent as one goes from one spectrum to another.

The Ether Linkage (–C–O–CH_3) Stretching Vibration.—Table II presents a comparison of the positions of the bands associated with the ether linkage[18] for the ten compounds studied which contained the methoxy group. No strong band occurs at this position in either 4-cholesten-3-one or *i*-cholestan-6-one, the two steroids in this study which contain no ether linkage. Also the spectrograms published by Furchgott[4] of dehydroisoandrosterone and androstenediol-3β-17α, which are the free alcohols of two of the methoxy compounds studied in this paper, do not have the ether linkage and do not show a major band in this region. It is possible to locate this vibration band in both the *i*- and the *n*-steroids in the solid state at about 1100 cm.$^{-1}$ even though there is overlapping with other bands yet to be discussed. The wave number of this band in solution is very constant, the position for the *i*-steroids, 1098 cm.$^{-1}$, being consistently 5 cm.$^{-1}$ to lower frequencies than that for the *n*-steroids, 1103 cm.$^{-1}$. It is thus possible to distinguish between *i*- and *n*-steroid methyl ethers by a study of this one spectrum band in

TABLE II

Compound	Frequency in cm.$^{-1}$ Solid	Solution CS_2
Stigmasteryl methyl ether	1100	1103
3β-Methoxy-5-pregnen-20-one	1095	1103
3β-Methoxy-5-androsten-17-one	1098	1103
3β-Methoxy-5-androsten-17β-ol	1085 (broad)	1103
Cholesteryl methyl ether	1104	1103
i-Stigmasteryl methyl ether	1098	1099
6-Methoxy-*i*-pregnan-20-one	1096	1098
6-Methoxy-*i*-androstan-17-one	1096	1098
6-Methoxy-*i*-androstan-17β-ol	1075 (very broad)	1098
i-Cholesteryl methyl ether	1101	1099

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(18) It is well known that the stretching vibration of the C–O–C linkage is responsible for a strong absorption band in the 1250–1110 region (8 μ to 9 μ). (See for example Randall, Fowler, Fuson and Dangl, ref. 12, p. 8.)

solution. It may be of interest to associate these different band positions with the different properties of normal and *i*-cholesteryl ether.[3]

The COH Group.—The medium weak bands at 1030 ± 1 cm.$^{-1}$ and 935 ± 1 cm.$^{-1}$ are present in both alcohol derivatives of methoxyandrostane. They are not present in the other steroids studied (with the exception of a 1031 cm.$^{-1}$ band in 3β-methoxy-5-androsten-17-one and a 932 cm.$^{-1}$ band in 4-cholesten-3-one). These bands may be associated with the deformation vibration of the OH group and the stretching vibration of the C–O bond, although more model compounds are needed to be certain.

Furchgott[4] discusses the 1070–1045 cm.$^{-1}$ region in connection with C–(OH) stretching vibrations in various configurations. For the two steroids having an OH attached to C_{17}, we found a major band *ca.* 1070 cm.$^{-1}$. But the two pregnane derivatives studied, which have no OH group, have medium bands at the same positions. Furthermore, 3β-methoxy-5-androsten-17-one has a major band at 1056 cm.$^{-1}$ Because of these new facts, we believe that one must be extremely cautious in making assignments in this region.

Influence of the Presence of a Ring Carbonyl.—With the exception of 4-cholesten-3-one, a band of medium intensity at 1007 ± 2 cm.$^{-1}$ is present in all of the steroids containing a ring carbonyl group. In contrast to this, the adjacent weak band at 998 ± 4 cm.$^{-1}$ is found only in the eight steroids having no ring carbonyl group. This 998 cm.$^{-1}$ band may be due to a CH bending vibration which is shifted to 1007 cm.$^{-1}$ in the presence of a carbonyl group.

Bands Present in Methoxy *i*-Steroid Compounds.—The weak bands at 1326 ± 4 cm.$^{-1}$, 1203 ± 6 cm.$^{-1}$, the medium weak band at 894 ± 3 cm.$^{-1}$ and the medium band at 861 ± 2 cm.$^{-1}$, are present only in the *i*-steroid compounds containing the 6-methoxy substituent.

Bands Present in All the Compounds.—The weak band at 1115 ± 4 cm.$^{-1}$ is present in *all* of the steroids studied. The weak bands at 1192 ± 4 cm.$^{-1}$ and 945 ± 6 cm.$^{-1}$ are present in all but *i*-cholestan-6-one, and the weak band at 835 ± 4 cm.$^{-1}$ is present in all except *i*-stigmasteryl methyl ether. The band at 1019 ± 5 cm.$^{-1}$, which is present in all the compounds but cholesteryl methyl ether and *i*-cholestan-6-one, is relatively more intense in the *i*-steroids than in the *n*-steroids.

Bands Related to Some Special Configurations.—In his attempt to correlate characteristic bands with certain structural configurations in steroid compounds, Furchgott[4] states that a band *ca.* 870 cm.$^{-1}$ arises from some vibration of the group composed of a carbonyl at C_3 conjugated with C_4=C_5. The spectra we obtained in this region are, with one exception, in agreement with this rule. 4-Cholesten-3-one, the only representative with this particular conjugated steroid, has a medium band at 870 cm.$^{-1}$. The spectra are devoid of bands in this region for all the rest of the steroids studied with the exception of *i*-cholestan-6-one which shows a medium band at 873 cm.$^{-1}$. This is a further confirmation of the similarity of influence of a cyclopropane ring and a carbon–carbon double bond.[8,9]

The 1400–1000 Cm.$^{-1}$ Region in Androstane Derivatives.—In contrast to the other steroid compounds, the androstane derivatives studied show many differences between solid and solution spectra, the more striking of which are listed in Table III. The bands at about 1143 cm.$^{-1}$ in the solid sample of the two alcohols shift about 9 cm.$^{-1}$ to lower frequency in solution. This shift is observed only in the case of the steroids having an OH group.

TABLE III

Compound	Frequency in cm.$^{-1}$	
	Solid (oil paste)	Solution in CS_2
3β-Methoxy-5-androsten-17β-ol	1312 Mw	......
	1148 M	1139 M
	1085 S broad	1103 S
	1067 w shoulder	
6-Methoxy-*i*-androstan-17β-ol	1324 Mw	......
	1250 M	1247 vw
	1138 Mw	1030 Mw
		1098 S
	1087-1064 VS very broad	1086 w
3β-Methoxy-5-androsten-17-one	1104-1100 S very broad	1103 S
	1073 w	1073 vw
6-Methoxy-*i*-androstan-17-one	1096 S	1098 S
	1085 S	1085 w
	1072 M shoulder	1073 vw

As has already been mentioned, Table II, the 1085–1075 cm.$^{-1}$ bands in the solid spectra shift 17 cm.$^{-1}$ to higher wave numbers in solution. In addition, that of the abnormal alcohol becomes much sharper. The bands at 1325 cm.$^{-1}$ and 1250 cm.$^{-1}$ in the abnormal alcohol and the 1312 cm.$^{-1}$ band in the normal alcohol are strong in the solid spectra and weak or non-existent in that of the solutions. The changes in the corresponding ketone derivatives are not so numerous, the major change being the disappearance of the 1087 cm.$^{-1}$ solid spectrum bands in the solution spectra. To account for the disappearing bands it may be concluded that strong intermolecular forces are present in these compounds in the crystalline state.[19]

Differentiation Between Normal and *i* Steroids by Means of Skeletal Vibration Bands

One of the most interesting structural differences between normal and *i*-steroids, aside from the fact that the substituents are located at different points, is the presence in the *i*-steroid skeleton of a modified cyclopropane ring. The theoretical interest of this strained ring has been recently discussed.[20,21]

The two fundamental skeletal deformation vibrations of cyclopropane are the symmetric vibration of the 3-carbon ring appearing at 1189 cm.$^{-1}$ and the corresponding asymmetric vibration usually assigned to the band at 868 cm.$^{-1}$.[22]

In cyclopropane derivatives both these vibrations are infrared active but the former falls in a region (*ca.* 1000 cm.$^{-1}$) full of bands in steroids and thus

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[19] T. A. Kletz and W. C. Price, *J. Chem. Soc.*, 644 (1947).

[20] A. D. Walsh, *Trans. Faraday Soc.*, **45**, 179 (1949).

[21] C. A. Coulson and W. E. Moffitt, *Phil. Mag.*, 7th Series, **40**, 1 (1949).

[22] G. Herzberg, "Infrared and Raman Spectra of Polyatomic Molecules," D. Van Nostrand and Co., Inc., New York, N. Y., 1945, p. 352. This assignment has been questioned by D. W. Scott, *et al.*, THIS JOURNAL, **72**, 4664 (1950).

not very useful for differentiation purposes. From Lespieau's[23] and Derfer's[24] data on the Raman and infrared spectra of substituted cyclopropanes it is possible to pick out a series of medium bands in the range 878–840 cm.$^{-1}$. Cleveland[25] lists 842 cm.$^{-1}$ and 879 cm.$^{-1}$ as very strong bands of 1,1-dimethylcyclopropane and spiropropane, respectively. It becomes of interest, therefore, to search the region between 900 cm.$^{-1}$ and 830 cm.$^{-1}$ to see if any such characteristic band might be found in the *i*-steroids which does not have a counterpart in the normal steroids.

The medium weak band at *ca.* 894 cm.$^{-1}$ and the band of medium intensity at *ca.* 861 cm.$^{-1}$ have already been mentioned as being present in all the five *i*-steroids containing a methoxy group. The sixth *i*-steroid studied, *i*-cholestan-6-one, has bands of comparable intensity at slightly higher frequencies than each of these, namely, at 903 cm.$^{-1}$ and 873 cm.$^{-1}$. If these two bands are included with the other sets of bands, either of these two band sets might be considered as a candidate for the cyclopropane ring deformation vibration. More model compounds are being studied in order to see whether or not this tentative assignment will prove significant.[26]

Acknowledgments.—We would like to express our appreciation to Dr. Percy L. Julian, Director of Research and Manager of Fine Chemicals, The Glidden Company, Soya Products Division, Chicago, Illinois, for suggesting the interest of this problem. We are indebted to Dr. Julian and his associate, Dr. Edwin W. Meyer, for supplying us with all the compounds reported, and for several interesting discussions during the course of the research. We wish to thank Dr. Jean Lecomte who, in the initial stages of this problem, gave one of us guidance and hospitality in his laboratory at The Sorbonne, Paris. We want also to acknowledge the technical assistance of Mr. Brandt Kuperstock and Mr. Robert Powell. This investigation was supported by a research grant from the National Cancer Institute, U. S. Public Health Service.

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[23] R. Lespieau, Bourguel and Wakeman, *Bull. soc. chim., France*, **51**, 400 (1933).

[24] J. M. Derfer, E. E. Pickett and C. E. Boord, THIS JOURNAL, **71**, 2482 (1949).

[25] F. F. Cleveland, M. J. Murray and W. S. Gallaway, *J. Chem. Phys.*, **15**, 742 (1947).

[26] In a private communication, dated 2/22/51, Dr. D. H. R. Barton of Birkbeck College Research Laboratory, the University of London, states that he has assigned the 1010 cm.$^{-1}$ band in the infrared spectrum of *i*-cholestane to a cyclopropane ring vibration. Our results (discussed above in the paragraph entitled "Bands present in all the compounds") do not contradict this assignment for the 1019 ± 5 cm.$^{-1}$ band especially since the one *i*-steroid exception, *i*-cholestanone, has a medium weak band nearby at 1007 cm.$^{-1}$. This *ca.* 1000 cm.$^{-1}$ band should therefore be taken into consideration also. It could not be used alone, however, to distinguish between normal and *i*-steroids because the former also have a band, sometimes equally intense, in this region.

NASHVILLE, TENNESSEE RECEIVED FEBRUARY 7, 1951

[CONTRIBUTION FROM THE RESEARCH LABORATORY OF ORQUIMA S. A., SÃO PAULO, BRAZIL]

α,γ- and β,γ-Dipyridyl

BY P. KRUMHOLZ

The dipyridyl, previously reported as the β,γ-isomer, has been shown to be α,γ-dipyridyl. It seems probable, on the basis of spectroscopic and electrochemical evidence, that the first structure is to be assigned to a base previously isolated from the products of the thermal decomposition of pyridine.

Studying the properties of isomeric dipyridyls[1] we found that the behavior of β,γ-dipyridyl, first isolated by Smith,[2] does not correspond to the assumed constitution. The second basic dissociation constant of Smith's dipyridyl is near to that of the α,β-isomer but about a hundred times smaller than that of β,β'- or γ,γ'-dipyridyl.[1] Smith based the constitution of his dipyridyl on the isolation of nicotinic and isonicotinic acid from the products of the permanganate oxidation of the dipyridyl. We decided therefore to repeat this oxidation under carefully controlled conditions.

The dipyridyl in question was prepared from a pure picrate (m.p. 215/216°) obtained by fractional crystallization of the picrates from the product of thermal decomposition of pyridine.[3] The picrate was decomposed by potassium hydroxide, the free base extracted with ether and after evaporation of the solvent recrystallized twice from hexane. The m.p. of the dipyridyl was 60.8–61.3°; two more crystallizations from hexane raised the m.p. to 61.1–61.5°. It was free of α,α'-dipyridyl as shown by the absence of the very sensitive color reaction with ferrous salts and could contain only traces of the liquid α,β-isomer, as on addition of 10% of the latter melting begins at 51°, being completed at 57.5°.

Five hundred mg. of the dipyridyl m.p. 60.8–61.3° was boiled under reflux with a solution of 4 g. of potassium permanganate in 100 ml. of water until the color of the permanganate disappeared completely (3 to 6 hours). The filtered liquid was concentrated to about 10 ml., carefully neutralized with hydrochloric acid, using congo red paper as indicator, and evaporated on the steam-bath to dryness. The residue was triturated with 5 ml. of cold absolute ethanol, filtered, and washed with a few ml. ethanol. After evaporation an oily residue remained, which was dissolved in 5 ml. of water and precipitated at 40° by dropwise addition of a saturated aqueous solution of copper acetate, until further additions yielded no more precipitate. Any excess of copper acetate must be avoided, as it dissolves the copper salt of picolinic acid. After collection, washing with a little water and drying, 130 mg. of a violet, crystalline copper salt was obtained, easily identified as the copper salt of *picolinic acid* by its typical crystal form, its solubility in hot water and the formation of α,α'-dipyridyl by dry heating.[4] The identification was further confirmed by recovery of the free acid from the copper salt. The latter was decomposed in hot aqueous solution by hydrogen sulfide, the filtered solution evaporated to dryness and the residue recrystallized from benzene. The m.p. of the acid was 135–136°. A mixture with an authentic sample of picolinic acid (m.p. 136–137°)

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[1] P. Krumholz, THIS JOURNAL, **73**, 3487 (1951).

[2] C. R. Smith, *ibid.*, **46**, 414 (1924).

[3] P. Krumholz, *Selecta Chimica*, **8**, 1 (1949).

[4] F. Blau, *Monatsh.*, **10**, 375 (1889).