Conversion of the Dimer of 2-Pyridone to the Dimer of N-Methyl-2-pyridone.—A mixture of 1.0 g. of the dimer of 2-pyridone, 50 ml. of water, 10 ml. of dimethyl sulfate and 4 g. of sodium hydroxide was stirred at room temperature for 6 hours and then filtered to remove unreacted starting material. Extraction of the filtrate with chloroform, followed by drying of the extract over anhydrous sodium sulfate and evaporation to dryness, yielded a small amount of a colorless solid, m.p. 225–226°, identical with an authentic sample of the dimer of N-methyl-2-pyridone, as determined by a mixture melting point determination and by a comparison of infrared spectra.

N,6-Dimethyl-2-pyridone was prepared essentially as described by Adams and Schrecker.[16] Recrystallization from diethyl ether gave colorless crystals, m.p. 56°.

Anal. Calcd. for C_7H_9NO: C, 68.27; H, 7.37; N, 11.37. Found: C, 68.39; H, 7.34; N, 11.39.

N,6-Dimethyl-2-iminopyridine.—A mixture of 23 g. of 2-amino-6-methylpyridine and 34 g. of methyl iodide was warmed on a steam-bath until a violent exothermic reaction set in. After the reaction had subsided, the mixture was cooled and the hygroscopic solid so obtained was recrystallized from ethanol to give 28 g. (53%) of N,6-dimethyl-2-iminopyridine hydroiodide. The free base was prepared by dissolving the salt in 100 ml. of water and shaking vigorously for 15 minutes with thoroughly washed silver oxide, prepared from 32 g. of silver nitrate and 10 g. of sodium hydroxide. Filtration of the mixture followed by evaporation of the filtrate under reduced pressure yielded a green, viscous liquid which could not be induced to crystallize. It was characterized as a picrate, which upon recrystallization from ethanol melted at 162–163°.

Anal. Calcd. for $C_{13}H_{14}N_5O_7$: C, 44.33; H, 4.01; N, 19.88. Found: C, 44.50; H, 3.87; N, 19.80.

The hydrochloride of N,6-dimethyl-2-iminopyridine was obtained by addition of concentrated hydrochloric acid to the above filtrate prior to evaporation. Subsequent concentration under reduced pressure yielded hygroscopic needles, m.p. 246–250°, which were used directly in the irradiation experiments without further purification.

The structure of this material was confirmed by treatment with nitrous acid (under the conditions described above for the conversion of 2-aminopyridines to 2-pyridones), followed by conversion to the picrate, m.p. 128°. Mixture melting point determinations and comparison of infrared spectra confirmed that the product of diazotization was N,6-dimethyl-2-pyridone.

Acknowledgment.—We are greatly indebted to Professor John D. Roberts and to Dr. James Burdon of the California Institute of Technology for many helpful comments and for their lucid analysis of the problem of the anomalous n.m.r. spectrum of N,6-dimethyl-2-pyridone, and to Dr. (Mrs). E. Smakula Hand for stimulating discussions throughout the course of this work.

(16) R. Adams and A. W. Schrecker, *J. Am. Chem. Soc.*, **71**, 1180 (1949).

[CONTRIBUTION FROM THE LABORATORY OF CHEMISTRY OF NATURAL PRODUCTS, NATIONAL HEART INSTITUTE, NATIONAL INSTITUTES OF HEALTH, BETHESDA 14, MD.]

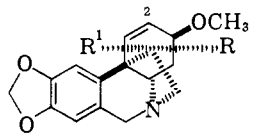
Intramolecular Hydrogen Bonding Studies with Semi-rigid Molecules. I. Derivatives of 5,10b-Ethanophenanthridine

BY H. M. FALES AND W. C. WILDMAN

RECEIVED AUGUST 16, 1962

Spectroscopic examination of the hydroxyl stretching frequencies of 32 compounds derived from the 5,10b-ethano-8,9-methylenedioxy-1,2,3,4,4a,5,6,10b-octahydrophenanthridine (crinane) nucleus has demonstrated the utility of this technique for the determination of hydroxyl configurations. Evidence is cited for the occurrence of interactions of hydroxyl groups with each other, with favorably situated alkoxyl, epoxy and carbonyl groups, and with the π-electrons of double bonds and aromatic rings. A useful technique for O-deuteration within the infrared cell is described.

Beyond the immediate goal of structural constitution, the study of natural products may yield unique series of compounds through which physical phenomena can be investigated and clarified. Detection of intramolecular hydrogen bonding by spectroscopic methods has proved to be particularly fruitful in structural studies of a great variety of complex organic compounds.[1–5] In previous papers on the alkaloids of the Amaryllidaceae, this technique provided a means of differentiating haemanthamine (I, R = OH, R′ = H) from epihaemanthamine (I, R = H, R′ = OH). The hydroxyl group in haemanthamine interacts with π-electrons of the C_1–C_2 double bond and in epihaemanthamine with those of the aromatic ring. Catalytic reduction of the isolated double bond caused a change in frequency of hydroxyl absorption in haemanthamine but not in epihaemanthamine.[6] The hydrogen bonding of vicinal methoxy alcohols within a semi-rigid ring system was discussed in connection with the structures of monotanine (II, R = OCH_3, R_1 = OH, R_2 = H) and coccinine (II, R = H, R_1 = OH, R_2 = OCH_3).[7] In the present paper, spectral studies are reported on oxygenated derivatives of the 5,10b-ethano-8,9-methylenedioxy-1,2,3,4,4a,5,6,-10b-octahydrophenanthridine (crinane) nucleus. The preparation and structures of these compounds have been presented in earlier papers of the Amaryllidaceae series.[8]



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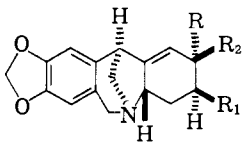


II

Experimental

A Beckman IR-7 spectrophotometer with prism-grating interchange was employed in all studies. Usually, a spectral slit-width of 5 cm.$^{-1}$ was used, furnishing an error in peak absorbance of less than 3%. The spectra were obtained by scanning at such a speed that tracking error was negligible. Most of the frequencies are considered accurate within 1–2 cm.$^{-1}$. The spectrophotometer was purged with dry air and calibrated against ammonia or water vapor in the region employed.

All substances were run in carbon tetrachloride in 0.1–5-cm. salt or silica cells under double-beam conditions. To ensure that intermolecular association was not occurring in samples showing more than one sharp band, the spectra were rerun at higher dilution (0.001–0.005 *M*) using a proportionately longer path length or proportionately increased ordinate scale. Insolubility became a severe problem with several of the diols, and it was necessary to

(1) L. P. Kuhn, *J. Am. Chem. Soc.*, **74**, 2492 (1952); **76**, 4326 (1954).

(2) (a) A. R. H. Cole and P. R. Jefferies, *J. Chem. Soc.*, 4391 (1956); (b) A. R. H. Cole, G. T. A. Müller, D. W. Thornton and R. L. S. Willix, *ibid.*, 1218 (1959); (c) A. R. H. Cole, P. R. Jefferies and G. T. A. Müller, *ibid.*, 1222 (1959); (d) A. R. H. Cole and G. T. A. Müller, *ibid.*, 1224 (1959).

(3) (a) P. von R. Schleyer, D. S. Trifan and R. Bacskai, *J. Am. Chem. Soc.*, **80**, 6691 (1958); (b) P. von R. Schleyer and R. West, *ibid.*, **81**, 3164 (1959); (c) P. von R. Schleyer, C. Wintner, D. S. Trifan and R. Bacskai, *Tetrahedron Letters*, **No. 14**, 1 (1959); (d) P. von R. Schleyer, *J. Am. Chem. Soc.*, **83**, 1368 (1961).

(4) R. Piccolini and S. Winstein, *Tetrahedron Letters*, **No. 13**, 4 (1959).

(5) R. L. Werner and B. M. Graham, *Australian J. Chem.*, **12**, 575 (1959).

(6) H. M. Fales and W. C. Wildman, *J. Am. Chem. Soc.*, **82**, 197 (1960).

(7) Y. Inubushi, H. M. Fales, E. W. Warnhoff and W. C. Wildman, *J. Org. Chem.* **25**, 2153 (1960).

(8) For a review of the alkaloids of this family, see W. C. Wildman in "The Alkaloids," Vol. VI, R. H. Manske, Ed., Academic Press, Inc., New York, N. Y., 1960, p. 289.

resort to both long path lengths and ordinate scale expansion. The use of low gain, relatively wide slits and slow scanning times (down to 1 cm.$^{-1}$/min.) permitted us to obtain satisfactory spectra.

Whenever an asymmetrical band or multiple bands were observed, the sample was deuterated[9] to eliminate the possibility of overtones or Fermi resonance. Samples were placed in silica cells, and a small drop of deuterium oxide was added to the top of the carbon tetrachloride solution. The spectrophotometer then was set to scan repeatedly until equilibrium was attained (1–2 hours). In all cases, the bands shifted to their expected positions around 2670 cm.$^{-1}$. The ratio ν_{OH}/ν_{OD} was very nearly constant (1.353–1.360) in all cases, indicating purely harmonic modes of vibration.

The frequencies are listed in Table I along with half-intensity band widths and integrated intensities (B-values)[10] per hydroxyl group. The whole band envelope was included in the latter measurement.

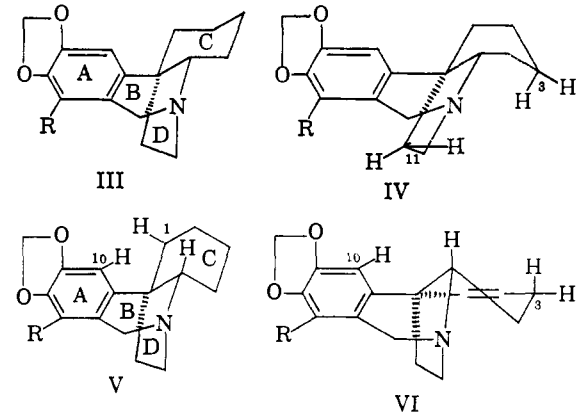
Structural conclusions and bond distances were derived from Cenco-Peterson atomic models.

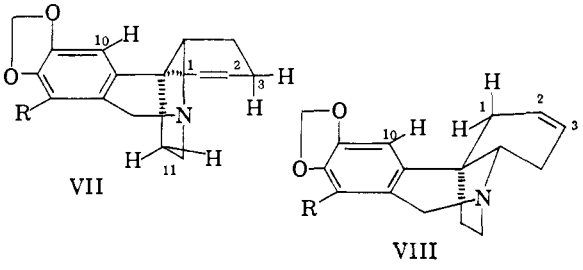
The authors gratefully acknowledge the assistance of Mrs. Katherine S. Warren in experimental aspects of this work.

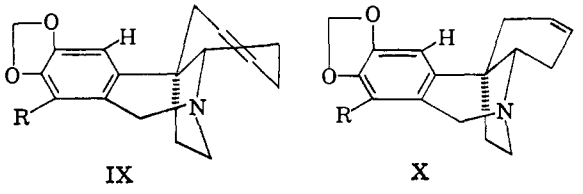
Discussion of Results

The crinane nucleus is particularly suited to conformational analysis since rings A, B and D constitute a rigid system. This limits the number and type of permissible conformations of ring C. Previous experimental data have proved the *cis*-C:D ring fusion.[11–16] One chair conformation (III) and two boat conformations (IV and V) are possible for this nucleus.[18] Both boat forms result in considerable steric interaction with more distant parts of the molecule. Cenco–Peterson models indicate the distance between the C_3- and C_{11}-hydrogen atoms in IV to be 1.06 Å., while in V the C_{10}-hydrogen is 1.14 Å. from one of the C_1-hydrogens.

When a C_1–C_2 double bond is introduced, the half-chair conformation VI renders substituents at C_3 either quasi-axial or quasi-equatorial. Only one half-boat form (VII) is possible. It is similar to IV except the C_3 substituents now have true axial and equatorial positions and the distance between the axial C_3-hydrogen and C_{11}-hydrogen is increased to 1.86 Å., thus relieving part of the interference associated with IV, while the hydrogens on C_1 and C_{10} are at a distance of 1.68 Å. If the double bond is moved to C_2–C_3, there is one half-chair conformation, VIII, in which the C_1-hydrogens again straddle the C_{10} aromatic hydrogen. Substituents at C_1 and C_4 become quasi-axial or quasi-equatorial. There also are two partial boat forms

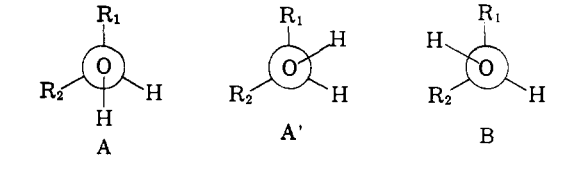






These cannot attain the half-boat conformation because of the *trans*-B:C ring fusion. Epoxy linkages at either C_1–C_2 or C_2–C_3 will result in conformations equivalent to VI–X.[19] Of these possible conformations for ring C, III, VI and VIII presumably are the most stable, and the results below are interpreted as arising only from chair or half-chair conformations of ring C.

Each conformation of ring C will provide a different environment for the hydroxyl hydrogen as it undergoes rotation about the C–O link. In the general case, three bands, not necessarily resolvable, may be expected in the hydroxyl stretching region corresponding to the three potential energy minima represented by rotational conformations A, A′ and B.[20]



Recently, attention has been called to the observation that saturated primary and secondary alcohols frequently possess two or more incompletely resolved

(9) This technique has also been applied to n.m.r. studies; *cf.*, H. M. Fales and A. V. Robertson, *Tetrahedron Letters*, **No. 3**, 111 (1962).

(10) $B = 2.303 \times 10^{-4}/Cl \int a d\nu$, where B is the apparent integrated absorption intensity, C is the molar concentration, l is the cell length in cm., and $\int a d\nu$ is the area of the band where absorbance is plotted *versus* frequency in wave numbers; *cf.*, D. A. Ramsay, *J. Am. Chem. Soc.*, **74**, 72 (1952).

Because of the low solubilities of many of these compounds in carbon tetrachloride, the intensities (B-units) are given to two places. In general, they vary from 0.34 to 0.43 B-unit per hydroxyl in the cases involving no hydrogen bonding. Bonding to an adjacent oxygen raises the intensity slightly [*cf.* P-XIVa (0.51) and P-XVIIa (0.57)] as does the presence of a *trans* axial group [P-XVa (0.57)].

Great care must be exercised in assigning configurations on the basis of intensity. Compounds XVIIIa and XVIIIe show identical intensities (0.42 B-unit) suggesting that the slight increase due to hydrogen bonding in the *cis* isomer is similar to the slight increase due to the inductive effect of the anti-parallel, axial methoxyl group in the *trans* isomer. Where a nearly linear hydrogen bond is achieved, as in the examples of 1,3-diaxial interactions (XXIII and XXV), there is a clear increase in intensity. Half-intensity band widths of the compounds studied range from 14–32 cm.$^{-1}$. The higher values generally are associated with band complexity, but exceptions to this were noted. Thus crinine (XIIa) exhibits a half-intensity band width of 30 cm.$^{-1}$ but no complexity, while the closely analogous powelline (P-XIIa) has a half-intensity width of only 21 cm.$^{-1}$. Crinamidine (P-XIIIa), although complex in this region, shows a width of only 19 cm.$^{-1}$.

(11) H. M. Fales and W. C. Wildman, *J. Am. Chem. Soc.*, **82**, 3368 (1960).

(12) P. F. Highet and W. C. Wildman, *J. Org. Chem.*, **25**, 287 (1960).

(13) H. M. Fales and W. C. Wildman, *ibid.*, **26**, 181 (1961).

(14) H. M. Fales and W. C. Wildman, *ibid.*, **26**, 881 (1961).

(15) E. W. Warnhoff and W. C. Wildman, *J. Am. Chem. Soc.*, **82**, 1472 (1960).

(16) Buphanitine [A. Goosen and F. L. Warren, *J. Chem. Soc.*, 1097 (1960)] was assigned a *trans*-C:D ring fusion on the basis of its degradation to a material resembling but not identical with (+)-powellane. A re-examination of rotational data [A. Goosen and F. L. Warren, *J. Chem. Soc.*, 4038 (1961)] has necessitated their retraction of this assignment, and these authors now concede that buphanitine is identical with nerbowdine.[17]

(17) *Cf.* ref. 13, footnote 19.

(18) For clarity and to provide continuity with formulas XI–XXIII, formulas III–X and XXIII and XXIV are drawn for the (+)-crinane nucleus. It should be pointed out that all alkaloids under discussion actually are derived from (−)-crinane.[11]

(19) B. Ottar, *Acta Chem. Scand.*, **1**, 283 (1947).

(20) These symbols follow the conventions of Oki and Iwamura.[21] The alcohols are viewed along the O–C axis and the oxygen atom is on the upper side. The circle indicates the carbon atom to which the hydroxyl group is attached.

(21) M. Oki and H. Iwamura, *Bull. Chem. Soc. Japan*, **32**, 306, 567, 950, 955, 1135 (1959); **33**, 427, 681, 717, 1600 (1960).

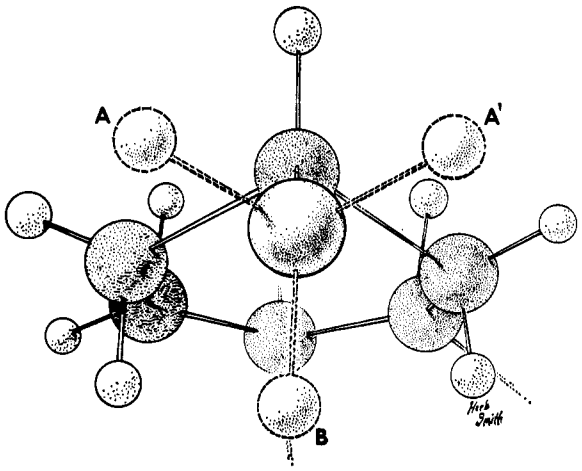


Fig. 1.

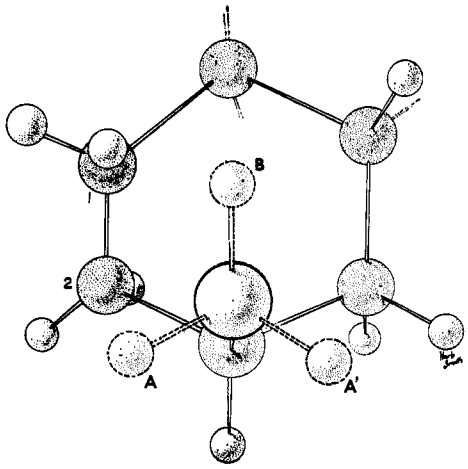


Fig. 2.

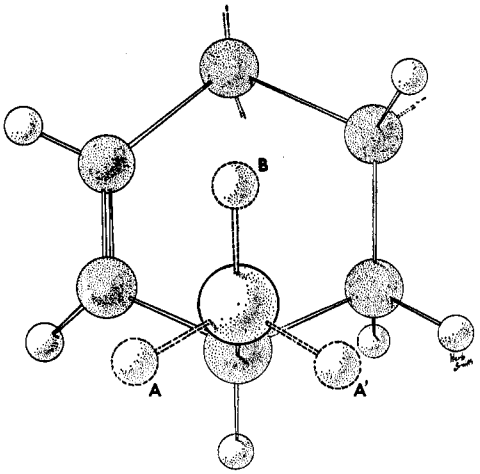


Fig. 3.

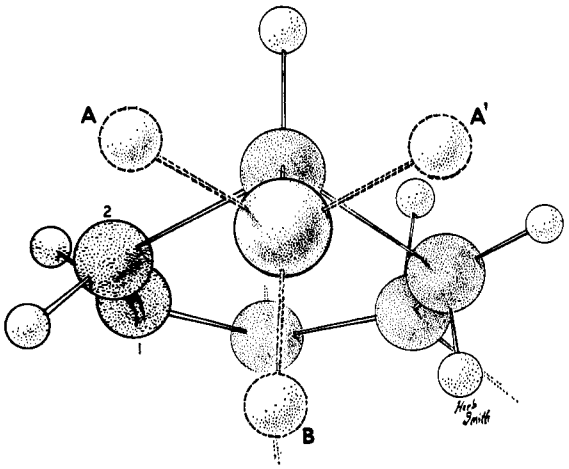
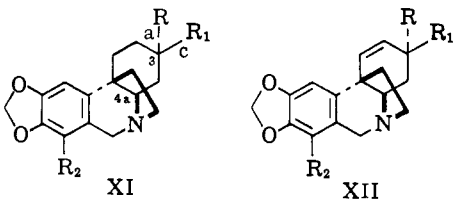


Fig. 4.

bands when examined under high resolution or in the overtone region.[4,5] Although Werner and Graham[5] ascribe the complexity to Fermi resonance with the adjacent $-CH_2-O-$ mode, Ōki and Iwamura have shown in an extensive series of investigations[21] that conformations A and A′ usually result in one band at 3626 cm.$^{-1}$ and conformation B in a second, smaller band at 3617 cm.$^{-1}$.[22] In addition, they have observed that when R_1, R_2 or both groups are properly oriented, unsaturated centers (allyl alcohol, diphenylmethylcarbinol), an interaction lowers the frequency of the species approximately 8 cm.$^{-1}$. The several bands may overlap considerably and their relative integrated intensities, other factors being equal, depend upon the relative number in each conformation at any instant. In turn, the proportion in each conformation depends both on steric factors and on hydrogen bonding; steric interference diminishes the population of a species and hydrogen bonding augments it.

Although an intramolecular hydrogen bond from a hydroxyl to an electronegative atom (O or N) usually causes a small increase in over-all integrated intensity, similar interaction with olefinic groups seems to have little effect on intensity.[5,7,23]

Nomenclature.—For simplicity, chemical names of compounds have been reduced to a minimum and numbers have been substituted. A "P" before the formula number (*e.g.*, P-XIa) indicates that the compound is in the powellane (R_2 = OCH_3) series rather than the crinane (R_2 = H) series which would be written XIa. Lower case "a" indicates axial or quasi-axial hydroxyl conformation while "e" denotes the equatorial or quasi-equatorial epimer. Unless designated to the contrary, ring C is considered to be in the chair or half-chair form. Formulas XI–XXIII are drawn to represent the compounds in their correct absolute configurations.



XI XII

1. **C_3-Monohydroxy Compounds.**—In the simplest cases, XIa, XIe, P-XIa and P-XIe, only one symmetrical band is observed at 3622–3629 cm.$^{-1}$ and is assigned to the free hydroxyl stretching mode. The axial epimers appear to absorb at slightly higher (5–6 cm.$^{-1}$) frequencies than do the equatorial isomers.[3a] The observed frequencies are in accord with a major contribution from rotational conformations A and A′ (Fig. 1). In the case of the axial epimers (Fig. 2), contributions from species B may be reduced by the additional interference from the axial C_1- and C_{4a}- hydrogens at a distance of 1.76 Å. Comparable interference is absent in the equatorial epimers, and there appears to be less

(22) A recent paper [F. Dalton, G. D. Meakins, J. H. Robinson and W. Zaharia, *J. Chem. Soc.*, 1566 (1962)] has discussed the doublet nature of the hydroxyl stretching frequencies in several monohydric alcohols. The conclusions drawn by these authors are essentially the same as those of Ōki and Iwamura, although no reference to the Japanese workers was made.

(23) G. C. Pimentel and A. L. McClellan in "The Hydrogen Bond," W. H. Freeman and Co., San Francisco, Calif., Reinhold Publishing Corp., New York, N. Y., 1960, p. 173.

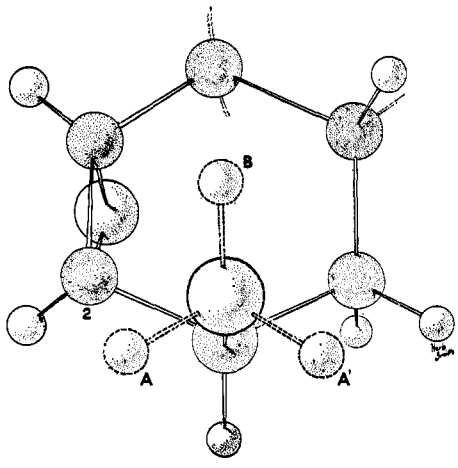


Fig. 5.

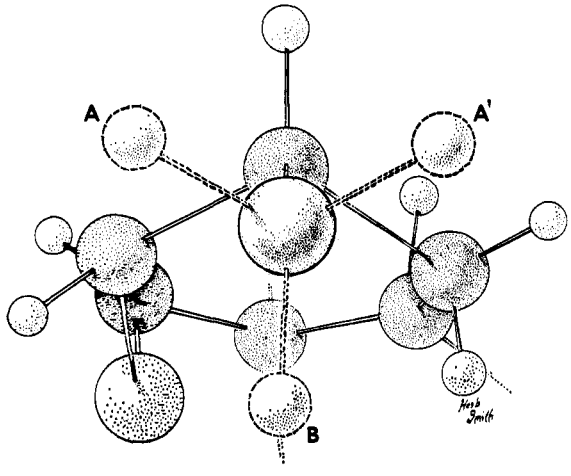


Fig. 6.

reason for favoring species A and A′ over B. Therefore, the very slightly lower frequencies at which the equatorial epimers absorb may reflect a slightly greater contribution from species B to the over-all curve even though no asymmetry is observed.[24]

In the allylic alcohols, it was noted that the quasi-axial isomers XIIa and P-XIIa absorb at *lower* frequencies than their quasi-equatorial counterparts XIIe and P-XIIe. Although this might be due to an inductive effect of the adjacent double bond, it is more likely that the frequency of species A (but not A′) in the axial epimers (Fig. 3) is lowered by 8 cm.$^{-1}$ due to interaction with the adjacent double bond. The population of this species presumably is increased over that of A′. The observed frequency (3620 cm.$^{-1}$) is, in fact, that of one of the bands of allyl alcohol, benzyl alcohol and diphenylcarbinol (3618–3619 cm.$^{-1}$).[21]

The quasi-equatorial isomers XIIe and P-XIIe (Fig. 4) show their main absorption at 3626 cm.$^{-1}$ which is assigned to corresponding species A and A′. In this case, neither species permits interaction of the hydroxyl hydrogen and the π-electrons of carbon 2 since the π-electrons are directed away from the hydroxylic hydrogen by the nature of the ring. However, the π-orbitals are within bonding distance of the hydroxyl group of species B. This gives rise to a pronounced shoulder at 3611 cm.$^{-1}$, again about 6 cm.$^{-1}$ lower than anticipated for an unperturbed species B. The absence of the second hydrogen on carbon 2 as well as the OH $\rightarrow \pi$ interaction may be responsible for the comparatively

(24) Ōki and Iwamura[21] resorted to graphical curve analysis to resolve complex spectra even when no asymmetry was obvious. Due to insolubility, many of our curves were taken under experimental conditions which did not warrant such an approach.

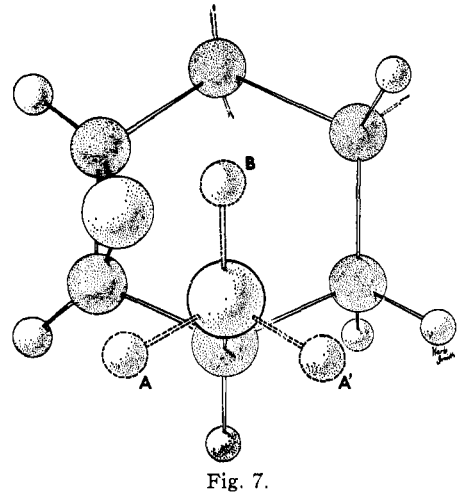


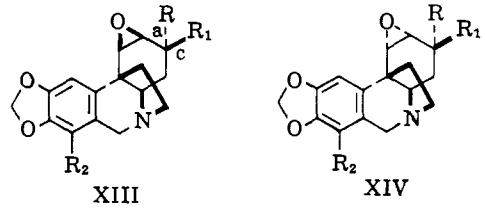
Fig. 7.

high intensity of the band. A band at 3610 cm.$^{-1}$ in methylvinylcarbinol is assigned to a similar species.[21]

A similar band from species B in XIIa and P-XIIa would be expected to be less visible due to the presence of the 3620 cm.$^{-1}$ band, and one is not observed.

It is unlikely that the shoulder at 3611 cm.$^{-1}$ is due to Fermi resonance or the presence of an overtone since the spectrum observed after deuteration of the hydroxyl group shows the same pattern at 2665 cm.$^{-1}$ (Table I).

If the double bond between carbons 1 and 2 is replaced by an epoxy group *cis* to the ethano bridge (as in XIII), hydrogen bonding from a *trans* quasi-axial C_3-hydroxyl to the epoxy oxygen is impossible, regardless of the conformation of ring C.



XIII XIV

Only a free hydroxyl group is observed in XIIIa and P-XIIIa at 3630 cm.$^{-1}$, indicating a large contribution from species A and A′ of Fig. 5. The shift of the main band to higher frequencies does expose a contribution from species B which is manifested as a slight broadening of the curve on the low frequency side. In these compounds the side of carbon 2 opposite the epoxy oxygen possesses no π-orbital with which the hydroxyl can interact. The slightly higher than normal frequency observed for species A and A′ probably reflects the polarity of the adjacent *trans*-epoxy oxygen; a similar effect was noted with XIVe and XVIIIa.

In the epimeric alcohol P-XIIIe, the main band is at 3599 cm.$^{-1}$ and probably represents species B (Fig. 6) where the hydroxyl hydrogen is weakly bonded to the adjacent epoxy group although the distance is rather long (2.8 Å.) compared to that of a more favorably oriented *cis* configuration which absorbs at 3575 cm.$^{-1}$ (see below). That this is indeed due to the bonded species of P-XIIIe (B, Fig. 6) is shown by the appearance of a shoulder at 3624 cm.$^{-1}$, the normal frequency for the unbonded species A and A′.

If the epoxy group is *trans* to the ethano bridge as in P-XIV, the quasi-axial hydroxyl of P-XIVa is *cis* to the adjacent epoxy group when ring C is in the half-chair form. This is a very favorable configuration for hydrogen bonding to the adjacent epoxy group in species A and B of Fig. 7, and only one band is observed at 3575 cm.$^{-1}$ which probably represents species A since it is

TABLE I

HYDROXYL STRETCHING FREQUENCIES

Compound	Symbol	Substitution R	Substitution R_1	Substitution R_2	Oxygen configuration assuming chair form of ring C C_1	C_2	C_3	Hydroxyl stretching frequencies (cm. $^{-1}$) (deuterated frequencies in paren.) Free OH	OH → π	OH → O	$\Delta\nu^1/_2$ (cm. $^{-1}$)	*B*-Units per hydroxyl
Dihydrocrinine	XIa	OH	H	H			a	3628			20	0.36
Dihydroepicrinine	XIe	H	OH	H			e	3622			18	.34
Dihydropowelline	P-XIa	OH	H	OCH_3			a	3629			15	.38
Dihydroepipowelline	P-XIe	H	OH	OCH_3			e	3624			15	.35
Crinine	XIIa	OH	H	H			a′		3620 (2670)		30	.36
Powelline	P-XIIa	OH	H	OCH_3			a′		3619		21	.43
Epicrinine	XIIe	H	OH	H			e′	3626 (2677)	3611sh (2665sh)		30	.41
Epipowelline	P-XIIe	H	OH	OCH_3			e′	3626	3611sh			.43
Flexinine	XIIIa	OH	H	H	(e)	(a)	a′	3630(skew)			20	.41
Crinamidine	P-XIIIa	OH	H	OCH_3	(e)	(a)	a′	3630(skew)			19	.42
Epicrinamidine	P-XIIIe	H	OH	OCH_3	(e)	(a)	e′	3624sh		3599	20	.44
Epoxypowelline	P-XIVa	OH	H	OCH_3	(a)	(e)	a′			3575		.51
Epiepoxypowelline	P-XIVe	H	OH	OCH_3	(a)	(e)	e′	3628				
Epidihydrobuphanamine	P-XVe	H	OH	OCH_3	e				3616		14	.34
Dihydrobuphanamine	P-XVa	OH	H	OCH_3	a			~3620sh	3599		23	.57
Demethoxyepidihydrobuphanamine	XVe	H	OH	H	e				3616		14	.34
Demethoxydihydrobuphanamine	XVa	OH	H	H	a			~3620sh	3602		24	.46
Epibuphanamine	P-XVIe	H	OH	OCH_3	e′				3614		13	.36
Buphanamine	P-XVIa	OH	H	OCH_3	a′				3584, 3613 (2644), (2655)			.40
Epoxybuphanamine	P-XVIIa	OH	H	OCH_3	a′	(e)	(a)			3568	32	.57
Epiepoxybuphanamine	P-XVIIe	H	OH	OCH_3	e′	(e)	(a)		3614		23	.46
α-Dihydroundulatine	XVIII	OH	H	OCH_3		a	a	3630, 3616sh			22	.42
Epi-α-dihydroundulatine	XVIII	H	OH	OCH_3		e	a			3582	20	.42
3-α-O-Tetrahydropyranyloxyisodihydrocrinamidine	XIX	..	..	OCH_3		a	a	3625(skew)			26	
3-β-O-Tetrahydropyranyloxyisodihydrocrinamidine	XIX	..	..	OCH_3		a	a	3628(skew)			27	
3-Tetrahydropyranyloxynerbowdine	XX	..	..	OCH_3	a		a			3526		
Dihydrocrinamidine	XXI	..	..	...	e		a	3626sh	3618			
Epinerbowdine	XXII	..	..	...	a		e	3625	3597			.30
Nerbowdine	XXIII	..	..	OCH_3	a		a	3623 (2674)	3581 (2645)	3533 (2611)		.93
cis-1,3-Dihydroxycrinane	XXIII	..	..	H	a		a	3625	3588	3536		
Oxonoerbowdine	XXV	..	..	...	a			3618 (2670)	3590 (2650)	3533 (2610)		.78

slightly less hindered than B where one 1,3-diaxial interaction is present.

In the epimer P-XIVe, bonding is impossible in any conformation of ring C. Consistent with this, only one band is observed at 3628 cm.$^{-1}$. This is slightly higher than the normal equatorial hydroxyl at 3622–3624 cm.$^{-1}$, perhaps again reflecting the electronegativity of the adjacent *trans*-epoxide oxygen. The hydroxylic hydrogen in A (Fig. 8) is 3.9 Å. from the epoxide oxygen.

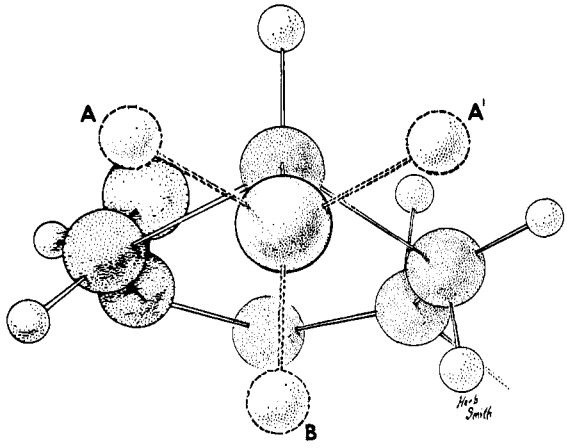


Fig. 8.

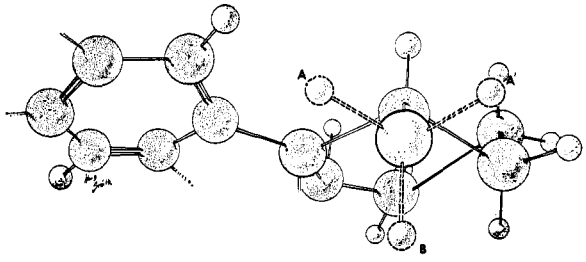
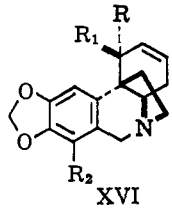


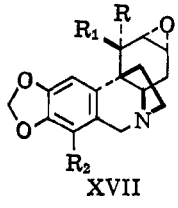
Fig. 9.

2. **C_1-Monohydroxy Compounds.**—Compound XVe possesses an equatorial hydroxyl (if ring C is in the

XV



XVI



XVII

chair form) and exhibits only one band at 3616 cm.$^{-1}$. It is suggested that this is due to species A (Fig. 9) which is slightly bonded to the adjacent benzene ring. Since this frequency is identical with that assigned to an unbonded species B, it would include any contribution from it. The axial isomer P-XVa (Fig. 10) exhibits its main band at 3599 cm.$^{-1}$. Initially this was thought to be due to the presence of the methoxyl at C_{10} which should be easily accessible for bonding by the C_1 hydroxylic hydrogen. However, this view is untenable because the corresponding demethoxylated compounds XVa and XVe exhibit bands at frequencies nearly identical with those found for P-XVa and P-XVe, respectively. The axial isomers of XV are even more favorably located for hydrogen bond formation with the benzene ring (A) than are the equatorial isomers. It is to this effect that the 3599 cm.$^{-1}$ band is ascribed. An extreme effect of this type has been noted previously in haemanthamine.[6] In this case, the frequency was lowered to 3560 cm.$^{-1}$ because the hydroxyl is situated on the ethano bridge in an even more favorable position for bonding to the benzene ring. A slight shoulder is

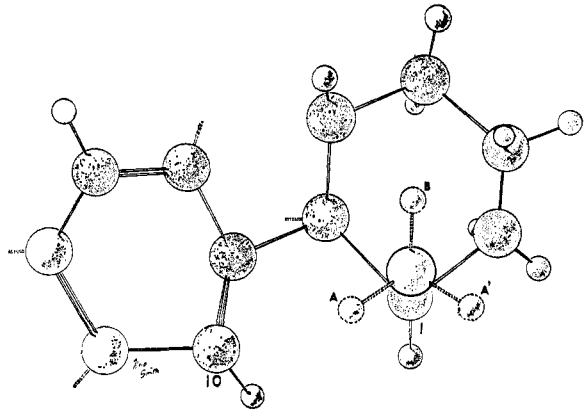


Fig. 10.

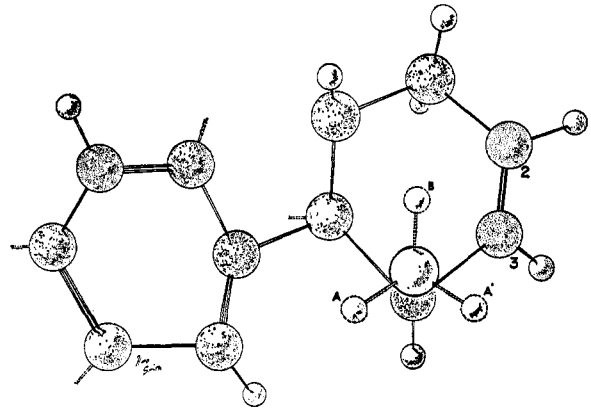


Fig. 11.

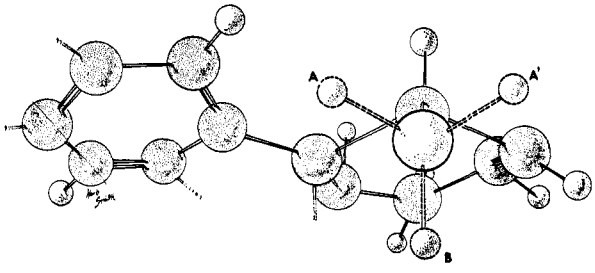


Fig. 12.

apparent in both P-XVa and XVa at 3620 cm.$^{-1}$ and presumably includes both unbonded species A′ and B (Fig. 10).

Introduction of a double bond at C_2–C_3 (P-XVIa) accentuates the strength of the hydrogen interaction with the benzene ring (A, Fig. 11) as shown by a frequency shift of the main band to 3584 cm.$^{-1}$ and causes the appearance of a new band at 3613 cm.$^{-1}$, characteristic of a species A′ or B hydroxyl interacting with the adjacent double bond. The corresponding quasi-equatorial isomer P-XVIe shows a band at 3613 cm.$^{-1}$, presumably due to the same ring-bonded species (A, Fig. 12) that is present in its fully equatorial counterparts, P-XVe and XVe. This band would include any contribution from species B which, by analogy with the simpler case (B of Fig. 4) would be expected to absorb at 3611 cm.$^{-1}$ However, it is not necessary to invoke species B to explain the observed band, since the epoxy analog of P-XVIe (P-XVIIe) exhibits the same band although it contains no species similar to B (see below).

Compound P-XVIIa is comparable to P-XIVa. The epoxy group is on the side of ring C opposite the ethano bridge, and the C_1-hydroxyl group occupies a quasi-axial conformation if ring C is in a half-chair form. Apparently the resultant bonding between the hydroxyl and

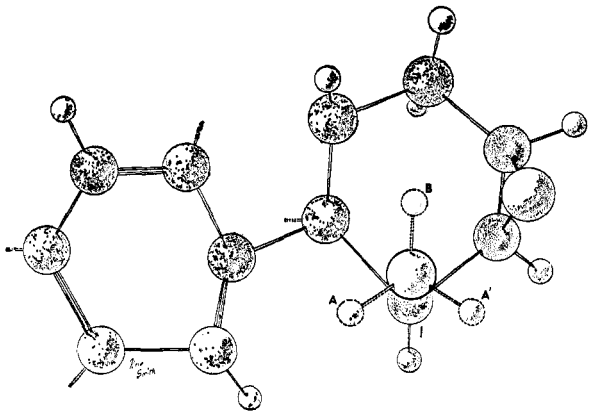
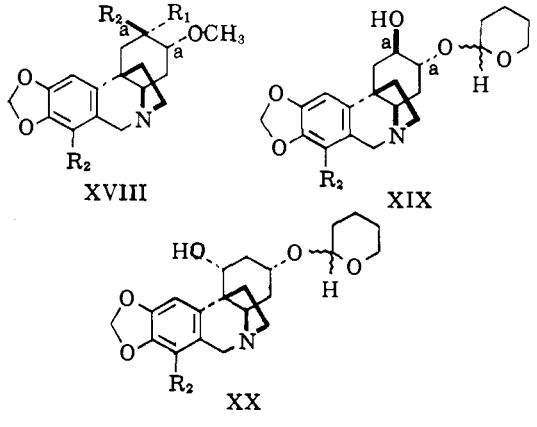


Fig. 13.

the epoxy group (A′ or B of Fig. 13) is stronger than the OH → π interaction (A) since only one band is observed at 3568 cm.$^{-1}$. The epimeric compound P-XVIIe (Fig. 14) cannot bond to the epoxy group in any conformation of ring C and exhibits only one hydroxyl band at 3614 cm.$^{-1}$, the same frequency as that of the corresponding quasi-equatorial allylic alcohol P-XVIe, and is also assigned to a weak interaction of species A (Fig. 14) with the benzene ring. Species A′, as in the case of A (Fig. 8), is too far away from the epoxide ring to interact (3.9 Å.).

3. Hydroxy Ethers.—α-Dihydro- and *epi*-α-dihydroundulatine[15] possess hydroxyl and methoxyl groups at positions C_2 and C_3. In α-dihydroundulatine these



XVIII XIX XX

groups have a *trans* diaxial configuration with ring C in the chair form. In support of this, the main band is observed at 3630 cm.$^{-1}$ and is assigned to species A and A′. The *trans* antiparallel methoxyl group appears to have caused a 4 cm.$^{-1}$ shift to higher frequencies. Probably because of this shift, a slight shoulder at 3616 cm.$^{-1}$ is discernible and assigned to species B, although the interference from the ethano bridge appears to be considerable. The epimer *epi*-α-dihydroundulatine contains an equatorial hydroxyl, shows only one band at 3582 cm.$^{-1}$ due to bonding with the adjacent methoxyl, and is assigned to species B exclusively.

In the analogous tetrahydropyranyl ethers (XIX) which also possess *trans* diaxial configurations at C_2 and C_3, bands were observed at 3625–3628 cm.$^{-1}$ which also were broadened toward low frequencies, indicating a contribution from a species analogous to B. The configuration at the α-carbon of the tetrahydropyranyloxy group had little effect.

In XX an especially favorable opportunity for 1,3-diaxial hydrogen bonding exists, and only one intense band at 3526 cm.$^{-1}$ is observed. This is the most intense and most strongly shifted band seen in the series.

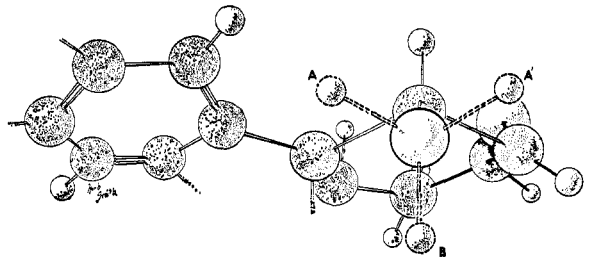
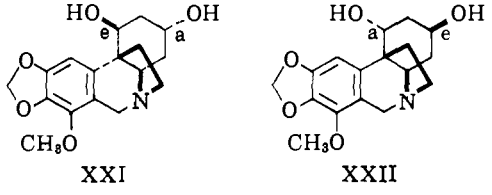


Fig. 14.

It falls near the frequency (3544 cm.$^{-1}$) assigned by Kuhn[1] to the corresponding vibration in *cis*-cyclohexane-1,3-diol (see below). The hydroxyl must be in conformation B.

4. Diols.—The 1,3-diols of this series provided especially interesting results. Compound XXI possesses *trans*-hydroxyl groups. Considering ring C in the chair form, the hydroxyl at carbon 1 is equatorial and that at carbon 3 is axial.



XXI XXII

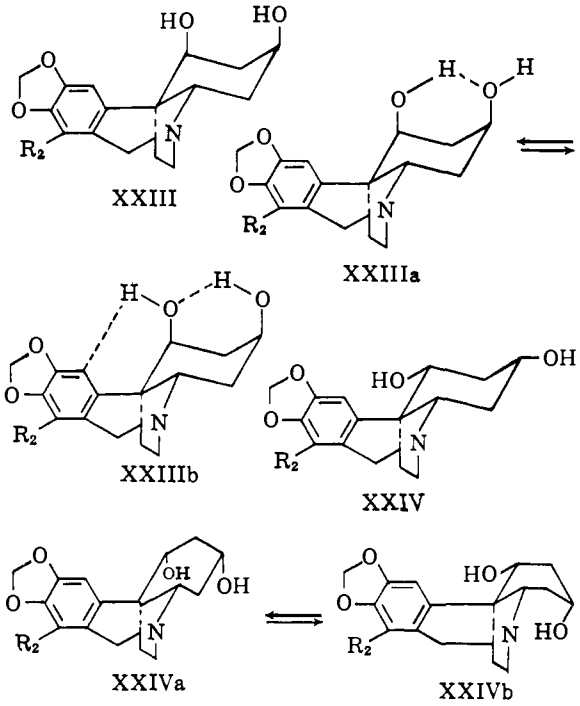
In agreement with this assignment, the main absorption is at 3618 cm.$^{-1}$ and is assigned to the same species of the C_1-hydroxyl involving participation with the aromatic ring as in XVe and P-XVe (Fig. 9). In addition, a shoulder at 3626 cm.$^{-1}$ is present and assigned to hydroxyl species A and A′ of carbon 3 (*cf.* Fig. 2). The 1,3-*trans*-hydroxyls cannot interact with each other in any conformation of ring C.

If the situation is reversed (as in XXII), stronger OH → π bonding to the aromatic ring becomes possible when ring C is in either the chair form or the boat form IV. Two distinct bands are observed. The one at 3625 cm.$^{-1}$ is assigned to the C_3-hydroxyl in species A and A′ (*cf.* Fig. 1) and that at 3597 cm.$^{-1}$ to the C_1-hydroxyl interacting with the benzene ring in species A by analogy with XVa and P-XVa (*cf.* Fig. 10). Since the chair form places the hydroxyl at C_3 in an equatorial instead of an axial position and also releases the flagpole interference associated with boat form IV, it would seem to be greatly favored in this case.

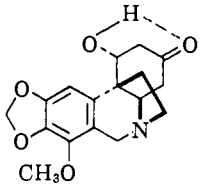
Nerbowdine has been formulated as a *cis*-1,3-dihydroxypowellane since it forms a cyclic O,O′-carbonate. Formation of this derivative does not allow a distinction to be made between isomers XXIII and XXIV because the latter may have undergone a conformational change to boat forms XXIVa and XXIVb. The first of these (XXIVa) possesses 1,3-diaxially oriented hydroxyl groups and would be expected to form a similar derivative.

The infrared spectrum of nerbowdine exhibits three bands. Absorption at 3623 cm.$^{-1}$ is accommodated by "free" hydroxyl groups[25] at C_3 in chair or boat forms of either XXIII or XXIV. Strong absorption at 3533 cm.$^{-1}$ is assigned to OH...O interactions between hydroxyls at carbons 1 and 3. As in the case of the cyclic carbonate, these facts do not allow a distinction to be made between the two isomers. However, the remaining band at 3581 cm.$^{-1}$ is in the vicinity of the frequencies found for the axial and quasi-axial hydroxyls of the analogous C_1-monohydroxy compounds XVa and P-

(25) Apparently an external hydrogen bonding to the oxygen of a hydroxyl group changes its frequency little, if at all; *cf.* ref. 3.



XVIa (3599 and 3584 cm.$^{-1}$, respectively) and may be assigned to the C_1-hydroxyl of XXIIIb. While conformation XXIVb permits the C_1-hydroxyl group to be within probable bonding distance of the aromatic nucleus, the actual distance appears to be considerably longer than that of XXIII and by analogy with XVe would give rise to a band near 3616 cm.$^{-1}$. In addition, conformation XXIVb would not exhibit 1,3-diaxial OH. . .O bonding at 3533 cm.$^{-1}$ nor would XXIVa exhibited the OH → π interaction mentioned above. Thus it becomes necessary to assume a comparable concentration of both conformations XXIVa and XXIVb in equilibrium in solution in order to explain partially the observed curve. However, the latter conformation (XXIVb) not only places the C_3-hydroxyl at a point in space occupied by a hydrogen atom on C_{11} of the ethano bridge but also causes loss of the powerful hydrogen bonding present in XXIVa. In contrast, all of the observed bands are neatly accommodated by the two rotational species XXIIIa and XXIIIb which do not require unlikely conformational changes of ring C.



Finally, XXV shows bands at 3618, 3590 and 3533 cm.$^{-1}$. The first two were expected by analogy with P-XVa or XXII. The last band is regarded as most unusual since it indicates that OH → carbonyl bonding is occurring although the distance is quite large (2.8 Å.).[26] It seems unlikely that the band is due to OH → π bonding with the carbonyl carbon because of its position and intensity which resembles that of the *cis*-diols XXIII and XXIII (no methoxyl). Such a bond also is unlikely considering the positive nature of the carbonyl carbon atom. Partial hydration of the carbonyl group was eliminated since the carbonyl band was unchanged on addition of a droplet of water or heavy water to the cell. Nor is the band an overtone of the carbonyl group[27] since the entire pattern was shifted to the 2700 cm.$^{-1}$ region on deuteration of the hydroxyl group. We are forced to conclude that hydrogen bonding is possible in axial 3-hydroxycyclohexanone systems.

(26) It is noteworthy that weak OH → O bonding is observed in P-XIIIe where the distance also is 2.8 Å.

(27) R. N. Jones and C. Sandorfy in "Technique of Organic Chemistry," Vol. IX, Interscience Publishers, Inc., New York, N. Y., 1956, p. 425.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY, ITHACA, N. Y.]

Evidence for Conformational Changes in α-Chymotrypsin-catalyzed Reactions. VI. Changes in Optical Rotatory Dispersion Parameters

BY BENT H. HAVSTEEN[1] AND GEORGE P. HESS[2]

RECEIVED JULY 23, 1962

The optical rotatory dispersion parameters of α-chymotrypsin, diisopropylphosphoryl-α-chymotrypsin and monoacetyl-α-chymotrypsin were determined at *p*H 3.8 and 12°. The dispersion parameters, a_0 and b_0, of monoacetyl-α-chymotrypsin and diisopropylphosphoryl-α-chymotrypsin were found to be similar but significantly different from the optical rotatory dispersion parameters of α-chymotrypsin. Deacylation of monoacetyl-α-chymotrypsin results in a molecule which has the same a_0 and b_0 values as α-chymotrypsin, indicating that the changes in the optical rotatory dispersion parameters are reversible and intimately related to the formation and breakdown of this enzyme-substrate compound. Studies of the *p*H dependence of the specific rotation, [α], at 365 mμ and of the temperature dependence of $[\alpha]_{365}$ at *p*H 3.8 and 4.0 also revealed significant differences between the enzyme-substrate compounds diisopropylphosphoryl-α-chymotrypsin, monoacetyl-α-chymotrypsin and α-chymotrypsin. Previous evidence, in combination with the data presented here, indicates that the formation of the enzyme-substrate compounds is accompanied by conformational changes.

Two reactions of α-chymotrypsin (CT) are considered in this investigation: the stoichiometric reaction of CT with diisopropylphosphorofluoridate (DFP) to give diisopropylphosphoryl-α-chymotrypsin (DIP-CT) and HF[3]; and the CT-catalyzed hydrolysis of *p*-nitrophenyl acetate.[4] This latter reaction proceeds *via* an intermediate, monoacetyl-CT, which can be isolated below *p*H 5.0.[5] Above *p*H 6.0, monoacetyl-CT, isolated according to the procedure of Marini and Hess,[6] is kinetically the intermediate in the chymotrypsin-

(1) Fulbright grantee, 1959–1962. This work is a small part of a thesis submitted by B. H. Havsteen to the Graduate School of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

(2) Address 1962–1963: Fulbright grantee and John Simon Guggenheim Fellow, Max Planck Institute for Physical Chemistry, Göttingen, Germany.

(3) E. F. Jansen, M. D. F. Nutting, R. Jang and A. K. Balls, *J. Biol. Chem.*, **179**, 189, 201 (1949); **185**, 209 (1950).

(4) B. S. Hartley and B. A. Kilby, *Biochem. J.*, **56**, 288 (1954); H. Gutfreund and J. M. Sturtevant, *Proc. Natl. Acad. Sci. U.S.*, **42**, 719 (1956); *Biochem. J.*, **63**, 656 (1956).

(5) A. K. Balls and H. N. Wood, *J. Biol. Chem.*, **219**, 245 (1956).

(6) M. A. Marini and G. P. Hess, *J. Am. Chem. Soc.*, **82**, 5160 (1960).