least reactive compound to bromine (which is likely to be α[13]), then with both polar and non-polar columns there is a definite peak, possibly the β-anomer, with a slightly greater retention time. With Apiezon L columns, Ferrier[21] assigned the middle of three peaks to β-ribose, having a longer retention time than the peak identified as α-ribose. However, as previously discussed, Ferrier's equilibrium mixtures were changed from the normal aqueous pattern owing to the preparative method used for the TMS derivatives; consequently, his "β" peak is considerably larger than the "α" peak. According to Cantor and Peniston,[28] 0.1 M ribose solution at pH 7 and 25° contains 8.5 mole % of reducible sugar (free aldehyde). Again, it is impossible to be certain of the anomeric and conformational assignments with the present information. Although solid ribose is usually regarded as the β-pyranose, Barker and Shaw[29] have cautioned against uncritical acceptance of this assignment. These workers also believe that β-ribose may favor the 1C rather than the C1 conformation.[31]

(31) NOTE ADDED IN PROOF.—After this manuscript was completed, the paper by Gee and Walker (*Anal. Chem.*, **34**, 650 (1962)) came to our attention, in which the chromatography of methylated trisaccharides is described.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK, PENNA.]

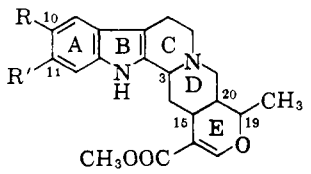
The Stereochemistry of the Heteroyohimbine Alkaloids[1]

BY MAURICE SHAMMA AND JANE MOSS RICHEY[2]

RECEIVED MARCH 15, 1963

The stereochemistry of the heteroyohimbine alkaloids has been elucidated. (1) The compounds can be assigned to their stereochemical groups by measurement of the chemical shifts of the C-methyl doublets of the free bases. An alternate procedure is by the study of the shapes of the infrared bands near 8.4 μ of the free bases. (2) The stereochemistry of the hydrogen at C-3 is defined by the nature of the peaks near 3.4 μ in the infrared. If the absorption is complex with at least two bands above 3.4 μ, the hydrogen is axial; if the absorption is simple, the hydrogen is equatorial. (3) The kinetics for the formation of the methiodide salts reflect the degree of steric hindrance at N_b. The allo-C-19α-methyl series methylates most slowly of any group. The allo-C-19β-methyl series, which undergoes a conformational change, methylates somewhat more slowly than the remaining groups. The pK_a values for the heteroyohimbine alkaloids generally show the same trend as the rates, although the differences are of a smaller magnitude. (4) The conformations of the methiodide salts can be determined by analysis of the chemical shifts of the $^{\oplus}$N-methyl groups. A *trans* C/D N-methylquinolizidinium system gives rise to a peak at about 3.3 p.p.m., whereas a *cis* C/D system shows a peak near 3.5 p.p.m. The raunitidine to isoraunitidine isomerization is thus shown to be an allo to epiallo transformation. (5) With the possession of members of the four possible groups in the allo and epiallo series it was possible by conformational analysis to assign the stereochemistry of the C-19 methyl groups. The assignment was also supported by a comparison of the C-19 methyl chemical shifts in the free bases and the corresponding methiodide salts and by the rates of methylation data. (6) The stereochemistry at C-19 in the normal series was determined by the study of the chemical shifts of the C-19 methyl groups.

The heteroyohimbine alkaloids share in common the skeleton I where R and R′ can be either hydrogen or methoxyl.[3] The first heteroyohimbine to be isolated, ajmalicine (R, R′ = H in I), was obtained in the course of the initial chemical investigation of *Rauwolfia*



I

serpentina by Siddiqui and Siddiqui.[4] In 1951, Goutarel and Le Hir proposed what has now come to be accepted as the correct structure for ajmalicine without dealing with the stereochemistry of the molecule.[5] The conclusions of the French school regarding the presence of the conjugated enol ether function were further confirmed by Bader, who compared the ultraviolet spectrum of ajmalicine with that of conjugated and non-conjugated enol ethers, and who also noted that the difficulty in hydrogenating the double bond of amjalicine is a characteristic property of enol ethers in general.[6]

With the isolation of reserpine and the subsequent rush toward the study of related indole alkaloids, a number of new heteroyohimbines were obtained so that to date sixteen members of this series have been recognized and are listed in Table I. These sixteen bases differ from each other either in stereochemistry or in the nature of the ring A substituent(s).

TABLE I

KNOWN HETEROYOHIMBINE BASES

Non-methoxylated: Tetrahydroalstonine, akuammigine, raunitidine, mayumbine, ajmalicine, and isoajmalicine

10-Methoxylated: Aricine and raumitorine

11-Methoxylated: Reserpinine, isoreserpinine, tetraphylline, raunitidine, and isoraunitidine

10,11-Dimethoxylated: Reserpiline, isoreserpiline, and rauvanine[7]

Possible Configurations and Conformations.—Before delving into the data which depict various details of the stereochemistry of the heteroyohimbine alkaloids, it is necessary to consider the configurations and conformations of the possible heteroyohimbine stereoisomers. If one considers only those isomers with an α-hydrogen at C-15, there are 2^3 or eight possible stereoisomers. There are in fact four basic stereochemical arrangements or configurations, namely, allo, epiallo, normal, and pseudo, and each of these may have the C-19 methyl group α or β, thus making for a total of eight stereoisomers.

The picture is further complicated, however, by the fact that a number of different conformations are possible for each of the four basic configurations. Thus if one considers only the conformations with the piperidine ring D in the more stable chair form, some of the more

(1) A preliminary account of this work was given in *J. Am. Chem. Soc.*, **83**, 5038 (1961), and **84**, 1739 (1962).

(2) Recipient of pre-doctoral fellowship No. GF-17,292 from the Division of General Medical Sciences of the National Institutes of Health.

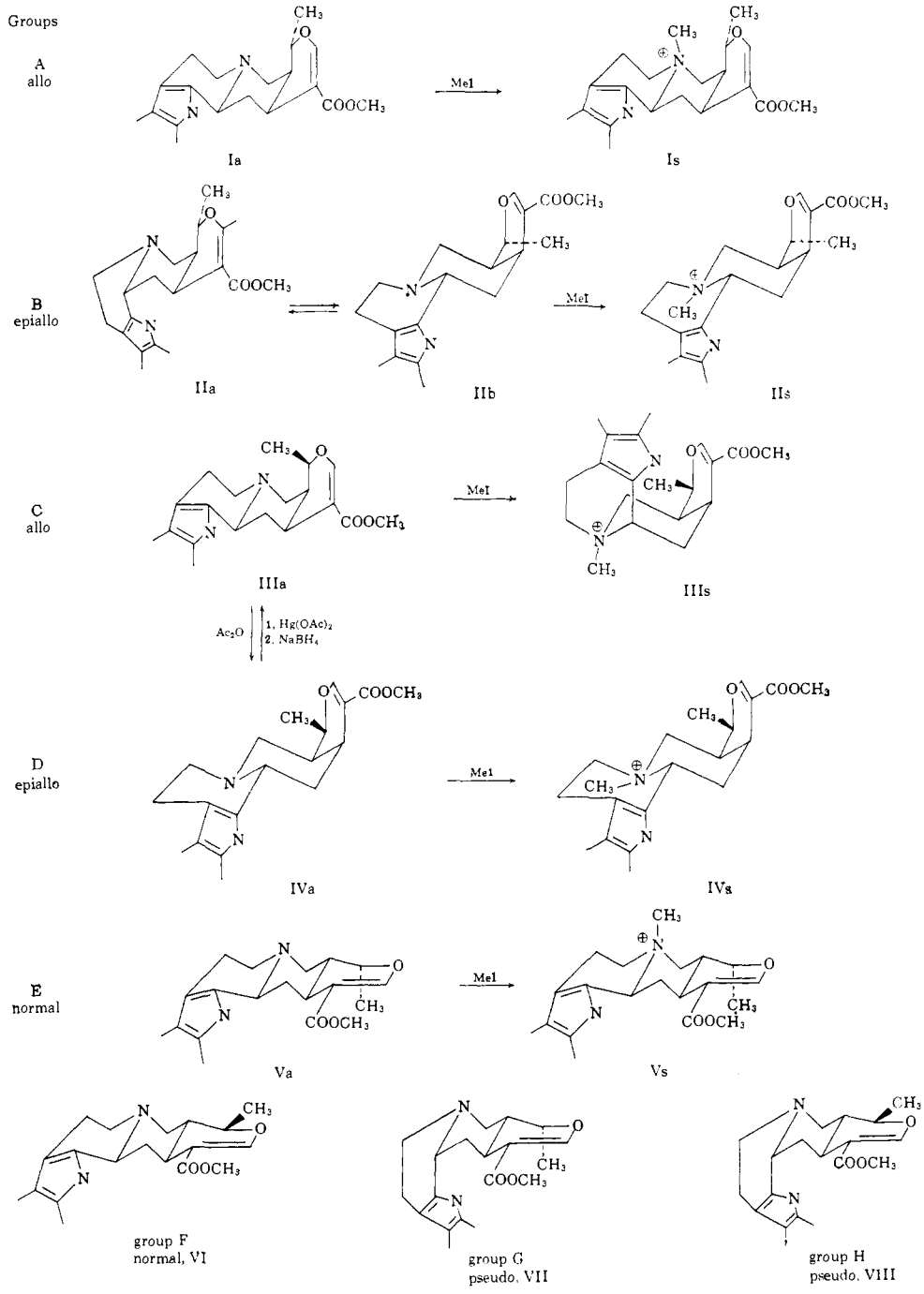
(3) For a review of the heteroyohimbine alkaloids see J. E. Saxton in "Alkaloids," Vol. VII, R. H. F. Manske, Ed., Academic Press, Inc., New York, N. Y., 1960, pp. 59–62.

(4) S. Siddiqui and R. H. Siddiqui, *J. Indian Chem. Soc.*, **8**, 667 (1931).

(5) R. Goutarel and A. Le Hir, *Bull. soc. chim. France*, **18**, 909 (1951).

(6) F. E. Bader, *Helv. Chim. Acta*, **36**, 215 (1953).

(7) R. Goutarel, M. Gut, and J. Parello, *Compt. rend.*, **253**, 2589 (1961)



important conformations possible for the free bases are Ia, IIa, IIb, IIIa, IVa, Va, VI, VII, and VIII.

It will be noted that an allo alkaloid will have a conformation such as Ia or IIIa, with the C-19 methyl group undefined at this stage, rather than a strained conformation such as IIIs (as the free base). An epiallo compound can exist either as IIa or IIb (C-19 methyl undefined), and as we shall see later the energy difference between these last two conformations is minimal.

Absolute Configuration at C-15.—The C-15α stereochemistry of ajmalicine was established by degradation of the alkaloid to dihydrocorynantheane. The same dihydrocorynantheane was then obtained from the 17,18-*seco* compound corynantheine, whose absolute configuration was known because of its correlation with dihydrocinchonamine. The absolute configuration at C-15 for the other heteroyohimbines is not so well defined; however, in analogy with indole alkaloids in general, it seems reasonable to assume that the C-15 hydrogen is always α, as pointed out by Wenkert.[8]

Stereochemical Classification of the Alkaloids.—Analysis of the n.m.r. spectra of the heteroyohimbine alkaloids at our disposal showed that the chemical shift of the C-19 methyl doublet at about 1.35 p.p.m. was very characteristic of the stereochemical group and was not dependent on the type of methoxy substitution in ring A. The values for the shifts are given in Table II.

In the few cases where the chemical shifts for different groups were nearly the same (*e.g.*, groups B and D) a second criterion based on infrared spectroscopy for differentiating between groups was used. Thus Neuss and Boaz have reported that the infrared bands for the heteroyohimbines near 8.4 μ in carbon disulfide solution are very characteristic of the stereochemical groups.[9]

(8) E. Wenkert and N. V. Bringi, *J. Am. Chem. Soc.*, **81**, 1474 (1959).

TABLE II

THE HETEROYOHIMBINE ALKALOIDS[a]

Groups and stereochemistry	Alkaloids	Free bases: C/D fusion, quinolizidine (from infrared near 3.4 μ)	Free bases: C-19 methyl chemical shift (J in c.p.s.)	Free bases: pK$_a$	Free bases: Rates of MeI formation × 10^4 sec.$^{-1}$	Methiodide salts: ^{a}N–CH$_3$ chemical shift	Methiodide salts: C/D fusion	Methiodide salts: C-19 chemical shift (and J in c.p.s.)	Methiodide salts: Δ p.p.m. C-19 methyl (base) − (salt)
A (Allo) C-19	Tetrahydroalstonine (I)	*trans*	1.38 (6.1)	5.83	1.03	3.42	*trans*	1.46 (5.8)	−0.08
CH$_3$: α and e	Aricine		1.37 (6.3)	5.75	1.21	3.43		1.47 (5.4)	− .10
	Reserpinine		1.38 (6.1)	6.01	1.36	3.39		1.48 (5.4)	− .10
	Isoreserpiline		1.39 (6.2)	6.07	1.51	3.41		...	...
B (Epiallo) C-19	Akuammigine (II)	*cis*	...	..	...	..	..	...	...
CH$_3$: α and e	Isoreserpinine		1.32 (6.5)	6.49	27.6	3.32	*trans*	1.39 (6.2)	−0.07
	Reserpiline		1.32 (6.3)	6.20	28.0	3.35		1.39 (5.7)	− .07
C (Allo) C-19	Rauniticine (III)	*trans*	1.42 (6.7)	6.24	16.3	3.50	*cis*	1.40 (6.5)	+ .02
CH$_3$: β and a	Raunitidine		1.42 (7.1)	6.20	18.5	3.50		1.41 (6.1)	+ .01
D (Epiallo) C-19	Mayumbine (IV)	*trans*	1.35 (6.3)	..	...	..	..	...	...
CH$_3$: β and e	Isoraunitidine		1.35 (6.8)	6.42	27.1	3.31	*trans*	1.43 (6.0)	−0.08
E (Normal) C-19	Ajmalicine	*trans*	1.16 (6.7)	6.31	23.5	3.35	*trans*	1.26 (6.2)	− .10
CH$_3$: α and a	Tetraphylline		1.16 (6.5)	6.39	26.6	3.36		1.26 (6.2)	− .10
F (Normal) C-19	Raumitorine[b]	*trans*	...	..	27.0	..	..	...	...
CH$_3$: β and e									

[a] All spaces left blank signify that insufficient samples were available for the measurements. [b] The C-3 hydrogen in raumitorine is α and axial as indicated originally by R. Goutarel and J. Poisson in J. Poisson's Docteur-es-Sciences thesis, "Recherches sur les Alkaloides des Racines du *Rauwolfia vomitaria*," Department of Pharmacy, University of Paris, 1959, p. 46.

Two of the new heteroyohimbine alkaloids at our disposal, rauniticine and raunitidine, were identical and unique in this region; raumitorine differed from any of the other heteroyohimbines, and isoraunitidine was very close to the rare mayumbine.

The analysis of the above mentioned region of the infrared coupled with the comparison of the C-methyl chemical shifts in the n.m.r. spectra allowed us, therefore, to classify the known heteroyohimbine bases into six distinct stereochemical groups (groups A to F). Two additional groups, G and H, examples of which were not at our disposal, should be added to this list to represent the pseudo series.

Stereochemistry at C-3.—An interesting method of studying the stereochemistry at C-3 is by means of the infrared bands between 3.4 and 3.7 μ. Wenkert's original contributions to this subject[10] were further refined by Bohlmann, who pointed out that the relative simplicity or complexity of these peaks is due to the equatorial or axial nature of the hydrogens α to the nitrogen in a quinolizidine system.[11] If there are two or more hydrogens in a *trans* diaxial relationship to the electron pair on the nitrogen a complex absorption is found in the 3.4–3.7 μ region. In the present work this idea was extended to examples in the heteroyohimbine series where the C/D system can be considered as a substituted quinolizidine. A C-3 axial hydrogen (C/D *trans*) gives rise to a complex spectrum, and a C-3 equatorial hydrogen (C/D *cis*) gives a simple absorption. However, before these findings could be published, Rosen[12] and Yamazaki[13] each published identical conclusions on the subject.

It has been shown that akuammigine (group B) is isomeric with tetrahydroalstonine (group A) at C-3.[3] The infrared spectra of the alkaloids of group B near 3.4 μ are relatively of a simple pattern, while those of tetrahydroalstonine and its analogs (group A) are complex, so that the bases belonging to the former group have to have a *cis* fused C/D system, and group B has to be *trans* C/D fused.

A similar study of the infrared spectra of ajmalicine (group E) and its 3-iso isomer isoajmalicine (group G) also demonstrated that ajmalicine and its ring A methoxy homolog tetraphylline must possess a *trans* C/D system, while isoajmalicine is *cis* fused. It can be concluded, therefore, that of the tetrahydroalstonine (group A) and ajmalicine (group E) pair one must be allo and the other normal; while for akuammigine (group B) and isoajmalicine (group G) one is epiallo and the other pseudo. At this stage, however, no definite choice could be made between these different possibilities.

All of the other heteroyohimbine stereochemical groups in our possession (groups C, D, and F) exhibited complex infrared absorptions near 3.4 μ indicating that only *trans* fused C/D systems were involved.

Stereochemistry of the D/E Ring Fusion for Groups A, B, E, and G.—Early work with rates of palladium–maleic acid dehydrogenation of the heteroyohimbines had led to the tentative assignment of a *trans* D/E ring junction to alkaloids of groups A and B, and a *cis* D/E ring junction to the bases in groups E and G.[14] In the present study, the pK$_a$'s for the heteroyohimbines showed a small but definite trend (see Table II) and the tetrahydroalstonine group (group A) was found to be the least basic of any group. If the differences in basicities between the various groups were to be interpreted as a steric effect, it seemed from the study of molecular models that a normal compound with a D/E *trans* system (Va) should be less hindered at N$_b$ and thus more basic than the allo analog with a *cis* D/E fusion (*e.g.*, Ia).

To investigate this point further, the rates of methiodide formation, in which the steric effects should lead to more marked differences than protonation, were studied. The pseudo-first-order rate constants for methiodide formation are given in Table II together with the corresponding pK$_a$ values determined in 68% dimethylformamide in water. The trend in the pK$_a$'s was generally the same as that of the rates, with the latter values showing much larger differences between groups. The tetrahydroalstonine group (group A) was found to methylate much more slowly than any other, indicating that this must be the group with the most hindered nitrogen. This slow rate can be explained only in

(9) N. Neuss and H. E. Boaz, *J. Org. Chem.*, **22**, 1001 (1957).

(10) E. Wenkert and D. K. Roychaudhuri, *J. Am. Chem. Soc.*, **78**, 6417 (1956).

(11) F. Bohlmann, *Ber.*, **92**, 1798 (1959).

(12) W. E. Rosen, *Tetrahedron Letters*, 481 (1961).

(13) F. Yamazaki, *Nippon Kagaku Zasshi*, **82**, 72 (1961); *Chem. Abstr.*, **56**, 10207*c* (1962).

(14) E. Wenkert and D. K. Roychaudhuri, *J. Am. Chem. Soc.*, **79**, 1519 (1957).

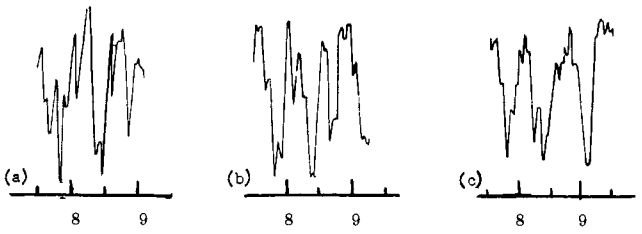


Fig. 1.—Infrared spectra in CS_2 of (a) rauniticine, (b) isoraunitidine, and (c) raumitorine.

terms of the allo configuration for that group, in which essentially an isopropyl moiety is in a 1,3-diaxial relationship to the pair of non-bonded electrons on N_b. Thus tetrahydroalstonine and the other members of group A must be allo with a D/E *cis* junction whereas the fast rates exhibited by ajmalicine and its analog in group E are compatible only with the normal configuration and a D/E *trans* ring junction. Because of the C-3 relationships, it now follows that group B must be epiallo and group G pseudo.

To confirm the use of rate constants in interpreting the D/E stereochemistry of the heteroyohimbines, rates were taken on various members of the yohimbine series where the configuration of the D/E ring junction was known. Thus yohimbine and corynanthine, which are known to be normal, exhibited faster rates of methylation than alloyohimbine or α-yohimbine which are allo (Table III).

TABLE III

YOHIMBINE TYPE ALKALOIDS

Alkaloids or derivatives	Stereochemistry	Rates of methylation $\times 10^4$ sec.$^{-1}$	Chem. shift of $^{+}N-CH_3$ protons, p.p.m.	C/D fusion of methiodides
Alloyohimbine	Allo	13.8	3.52	*cis*
α-Yohimbine	Allo	1.16	3.29	*trans*
Yohimbine	Normal	48.7	3.28	*trans*
Corynanthine	Normal	74.9	3.29	*trans*
Pseudoyohimbine	Pseudo	≈717	3.49	*cis*

Members of the epiallo group B exhibited a relatively fast rate of N-methylation. No conclusion could be drawn, however, at this stage as to whether it was conformation IIa or IIb that was methylating, since the energy difference between the two forms is minimal. Furthermore, three groups of alkaloids, namely, C, D, and F, could not have their stereochemistry elucidated even though their rates of methiodide formation were measured and group C (rauniticine) had been found to methylate slightly more slowly than the other two. Our attention, therefore, turned to the study of the raunitidine–isoraunitidine isomerization.

The Raunitidine–Isoraunitidine Isomerization.—Salkin and co-workers had reported, in 1961, the isolation of two new heteroyohimbine alkaloids, rauniticine and raunitidine, which from spectral and analytical data appeared to be, respectively, non-methoxylated and 11-methoxylated species.[15] Both compounds showed a complex absorption in the 3.4 μ region which indicated a *trans* C/D quinolizidine system. The absorption near 8.4 μ together with the C-methyl n.m.r. data showed that the two alkaloids belonged to a new stereochemical group. The Penick group also found that on treatment with acetic anhydride rauniticine was recovered unchanged, but raunitidine was partially converted to a new isomer, isoraunitidine.[15] The infrared spectrum of isoraunitidine was still complex at 3.4 μ, indicating the retention of the *trans* C/D quinolizidine system, while the region around 8.4 μ was almost identical with that shown by mayumbine so that the two alkaloids belonged to the same group.

When we further studied the raunitidine–isoraunitidine isomerization it became evident that the transformation was essentially irreversible since: (a) On heating raunitidine over a steam bath for 18 hr. in 13% acetic acid in acetic anhydride, and working up according to Salkin, a 4% yield of isoraunitidine was obtained and 94% of the starting material recovered. (b) When the heating period was extended to 4 days, the yield of isoraunitidine increased to 25%. (c) Unchanged isoraunitidine was recovered in 97% yield after heating in the above mentioned solvent for 18 hr. No raunitidine could be isolated.

A pseudo–normal isomerization is not involved here because the *trans*-quinolizidine system with the axial C-3 hydrogen is present in the starting material and the product. Hence the isomerization must proceed from an allo to epiallo form or *vice versa* and involves conformation IIIa (allo) and IVa (epiallo).

No criterion previously used in the yohimbine series was able to distinguish which of the two compounds in question was allo and which was epiallo. Katritzky and co-workers, however, had found that in a series of N-methylquinolizidinium cations, the ^{+}N–methyl protons of methiodides with *cis* fused rings absorb at lower fields than their *trans* fused analogs.[16] This difference was now also apparent in the yohimbine series where the n.m.r. spectra were taken of alkaloid methiodide salts with known C/D stereochemistry in the free bases. From Table III it can be seen that, for the series of yohimbine alkaloids, the $^{+}N-CH_3$ peak in a *trans* fused N-methyl quinolizidinium system appears near 3.3 p.p.m. while the corresponding peak in the *cis* fused series shows up near 3.5 p.p.m. Alloyohimbine undergoes the type of conformational change prior to methylation indicated by going from IIIa to IIIs (with ring E of the yohimbine type), while α-yohimbine methylates directly to give a *trans* fused N-methylquinolizidinium system (Ia to Is with ring E of the yohimbine type). This difference in the methylation paths of the two allo alkaloids is also reflected in the rates of methylation since alloyohimbine methylates more than ten times faster than α-yohimbine. The reason for this variation between two allo alkaloids becomes clear from a consideration of the ring E substituents. In alloyohimbine the carbomethoxyl group is equatorial and the hydroxyl group axial, and a change in conformation prior to methylation would only reverse this stereochemical order. In the more rigid α-yohimbine molecule, on the other hand, both substituents are equatorial, and a change in conformation would lead to two axial substituents; such a transformation, therefore, is not favored and α-yohimbine methylates directly at a very slow rate to give a *trans* C/D fused methiodide.

The n.m.r. spectrum of raunitidine methiodide showed an ^{+}N-methyl peak at 3.49 p.p.m. (*cis* N-methylquinolizidinium system) whereas the peak for isoraunitidine was further upfield at 3.31 p.p.m. (*trans* N-methylquinolizidinium system). Also, as noted previously, raunitidine (group C) methylated more slowly than isoraunitidine (group D). Hence raunitidine must be like alloyohimbine, undergoes a conformational change before methylation, and can be represented by IIIa while its salt has the form IIIs. Isoraunitidine must therefore be IVa and undergoes methylation directly to give a methiodide IVs with a *trans* fused C/D system. The conversion of raunitidine to isoraunitidine therefore involves an allo to epiallo isomerization, the

(15) R. Salkin, N. Hosansky, and R. Jaret, *J. Pharm. Sci.*, **50**, 1038 (1961).

(16) T. M. Moynehan, K. Schofield, R. A. Y. Jones, and A. R. Katritzky, *J. Chem. Soc.*, 2637 (1962).

first such isomerization found in the heteroyohimbine series. This conclusion, incidentally, is also in agreement with the fact that, in general, sodium borohydride reduction of 3-dehydro compounds with *cis* D/E rings leads predominantly to the allo configuration, and reduction of 3-dehydroisoraunitidine which is identical with 3-dehydroraunitidine has been found to give raunitidine rather than isoraunitidine.[15]

Turning back now to the epiallo alkaloids of group B, Wenkert has recently pointed out that these bases do not exhibit a strong n.m.r. peak for a C-3 equatorial proton near 4.5 p.p.m.[17] These data coupled with the infrared absorption near 3.4 μ (*cis* quinolizidine system) indicate that the bases of this group indeed exist as an equilibrium of conformations IIa and IIb. The N-methylation proceeded at a relatively fast rate, and it must be conformation IIb that methylates since the resulting methiodide salts exhibit an n.m.r. peak at about 3.33 p.p.m. characteristic of a *trans* fused N-methylquinolizidinium system.

The Stereochemistry at C-19 in the Allo–Epiallo Series.—With the elucidation of the stereochemistry at C-3, 15, and 20, of raunitidine and isoraunitidine, the four possible C-19 isomeric groups in the allo and epiallo series were now known: group A (tetrahydroalstonine) and C (rauniticine) are allo, and groups B (akuammigine) and D (mayumbine) are epiallo.

Considering the two groups of epiallo alkaloids, isoraunitidine (group D) exists in only one main conformation IVa while the members of group B exist as mentioned above in conformations IIa and IIb. This difference in preferred conformation between the two epiallo groups can be explained best in terms of the C-19 methyl configuration. It is reasonable to assume that the C-19 methyl group will choose to be in the favored equatorial position in each case, and that the epiallo compounds will prefer to assume that conformation which will accommodate the C-19 methyl group as an equatorial substituent. Thus group D has the C-19 methyl group β and equatorial, and group B has the C-19 methyl group α and equatorial in expression IIa. Consequently, groups A and C are assigned the 19α- and 19β-methyl configurations, respectively, since they are the C-3 diastereoisomers of groups B and D.

Further support for this assignment comes from two sources: (a) As can be seen from Table II, in all but two cases the C-19 methyl groups of the methiodide salts experienced a downfield n.m.r. shift of approximately 0.08 p.p.m. in relation to the free bases. The two exceptions were rauniticine and raunitidine (group C) for which a small upfield shift of about 0.015 p.p.m. was observed. The downfield shift in the salts can be explained by the deshielding of the C-methyl protons by the net positive charge on N_b. In groups C, however, the slight net upfield shift observed can be rationalized by an initial downfield shift in the free bases themselves due to the proximity of the C-19 methyl group to N_b which could lead to some hydrogen bonding. In fact, steric hindrance alone has been reported to cause a downfield shift in the n.m.r.[18] When the methiodide is formed, however, this strong steric interaction has vanished due to the conformational change, and the net result is a small upfield shift for the C-19 methyl doublet. (b) The allo alkaloids of group C methylate more than ten times faster than the allo bases of group A. This is in agreement with the assignment of the axial C-19 methyl group to the alkaloids of group C which can relatively easily change conformation from IIIa to give ultimately the salt IIIs with the C-19 methyl function now occupying an equatorial position. In the allo group A, however, the C-19 methyl is already equatorial so that a conformational change does not occur and methylation takes place directly but slowly because of 1,3-diaxial interaction with the incoming methyl iodide molecule. The N-methylation of the allo groups C and A, therefore parallel closely that of alloyohimbine and α-yohimbine, respectively, both in terms of rates of methylation and n.m.r. spectra of the methiodide salts.

Stereochemistry of the Normal and Pseudo Series, Groups E, F, G, and H.—While we had in our possession members of the four possible isomeric groups of the allo and epiallo series, in the normal and pseudo groups we had only three alkaloids, namely, ajmalicine, tetraphylline, and raumitorine.[19] The first two, as their infrared and n.m.r. spectra indicated, were in the same stereochemical group (group E), and raumitorine was in a different group, namely, F. All three alkaloids had a complex series of bands around 3.4 μ, so that the C/D quinolizidine system is *trans* fused as expected of the normal, but not the pseudo, series. The rates of methiodide formation as seen from Table II do not differentiate between normal groups with the C-19 methyl α or β (groups E and F). However, a conclusion about the stereochemistry at C-19 can be drawn from the n.m.r. chemical shifts of the C-methyl group. Ajmalicine and tetraphylline (group E) had their C-methyl peaks further upfield than any other heteroyohimbine. This upfield shift can be explained by assigning the α-axial configuration to the methyl substituent in group E, for this is the only case of an axial methyl group in the heteroyohimbine free bases, except for rauniticine and raunitidine (group C) which constitute a special case as mentioned previously. If the C-19 methyl group is α and axial in ajmalicine (Va), then it must be β and equatorial in raumitorine (VI) by a simple process of elimination.

While no examples of the pseudoheteroyohimbines were in our possession, these compounds can be related to either group E or F by epimerization at C-3, and isoajmalicine can therefore be represented by VII. The pseudo compounds would be expected to show a simple absorption at 3.4 μ in the infrared and an extremely fast rate of methylation due to only two 1,3-diaxial hydrogen interactions with the incoming methyl iodide molecule. Thus the combination of infrared spectroscopy and rate data should readily characterize a pseudo type heteroyohimbine.

Subsequent to the completion of this work and concurrent with our initial publication on this subject, Wenkert and co-workers published some elegant results also elucidating the stereochemistry of the heteroyohimbines.[17] Their results were in agreement with our own conclusions, although their reasoning was along completely different lines.

The elucidation of the structure of the heteroyohimbines settles the stereochemistry of alstonine (IX) and serpentine (X) which were known to be related to tetrahydroalstonine and ajmalicine, respectively. Very recently Fritz has published degradative and molecular rotational data which further indicate that serpentine must be X.[20] van Tamelen has also pointed out in 1961 at the 139th National Meeting of the American Chemical Society, held in St. Louis, Mo., that the D/E ring fusion of ajmalicine had to be *trans* on the basis of his total synthesis of this alkaloid.[21]

(17) E. Wenkert, B. Wickberg, and C. Leight, *J. Am. Chem. Soc.*, **83**, 5037 (1961), and *Tetrahedron Letters*, **No. 22**, 822 (1961).

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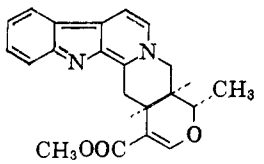
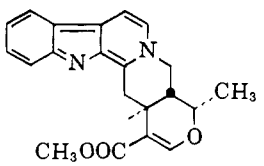
(21) E. E. van Tamelen and C. Placeway, *J. Am. Chem. Soc.*, **83**, 2594 (1961).



IX



X

Experimental

All melting points (uncorrected) were taken on a Nalge melting point block equipped with polarized light.

Sources of Heteroyohimbine Alkaloids.—Purified samples (approximately 200 mg. each) of the following heteroyohimbines were supplied by S. B. Penick and Co.: ajmalicine, tetraphylline, reserpinine, isoreserpinine, reserpiline, isoreserpiline, tetrahydroalstonine, aricine, raunitidine, isoraunitidine, and rauniticine. Reserpiline, being amorphous as the free base, was supplied as its oxalate salt and was regenerated by treatment with base. A further quantity of raunitidine was obtained from S. B. Penick and Co. so that the C-3 isomerization of this base could be studied.

Dr. Robert Goutarel of the Centre National de la Recherche Scientifique sent us further samples of reserpiline and isoreserpiline, a few milligrams of raumitorine, and twelve precious milligrams of mayumbine. The mayumbine sample was accompanied by the note: "The plant, *Pseudocinchona mayumbensis*, has been collected in Angola, and for obvious reasons it is impossible to procure it again."

Infrared Spectra.—All infrared spectra were taken on a Beckman IR5-A infrared spectrophotometer. The spectra were taken in chloroform as solvent, using concentrations of approximately 2% in a 0.5-mm. cell. The spectra in carbon disulfide were taken with solutions one-tenth as concentrated owing to the low solubility of the alkaloids, and a 5-mm. cell was used. The spectra of the 3-dehydroperchlorates were taken in disks made from potassium bromide which had previously been pulverized in a quartz mortar and pestle and dried under vacuum at 500°.

Nuclear Magnetic Resonance Spectra.—The nuclear magnetic resonance spectra of the alkaloids both as free bases and as methiodide salts were taken on a Varian Associates spectrometer, model 4300-2, with a fixed 40 Mc. radiofrequency unit. The spectrometer was equipped with a regulated power supply unit, a super-stabilizer, and a linear sweep unit. The spectra were recorded on a Varian Associates graphic recorder, model G-10, and were calibrated by imposing side bands with a Hewlett-Packard low frequency function generator, model 202A, for which the exact frequency was counted by a Hewlett-Packard industrial electronic counter, model 521-C.

The samples were prepared in 5-mm. precision-bore tubing. In the case of the free bases, 20 mg. of material was weighed into the sample tube and 0.2 ml. of deuteriochloroform was added to dissolve the solid, although in some cases excess solvent had to be added to effect solution. A drop of tetramethylsilane (TMS) was added as an internal standard. In the case of the methiodides, the salts were prepared by dissolving approximately 20 mg. of alkaloid in chloroform and adding a large excess of methyl iodide. After standing at room temperature for 24 hr., the solutions were evaporated to dryness under vacuum and the total product was then dissolved in a minimum amount of formamide (about 0.4 ml. usually) and transferred to an n.m.r. tube. Sufficient TMS could be dissolved in formamide to use this as an internal standard again.

The spectra were recorded five to eight times, sweeping the magnetic field in alternate directions. Side bands from the TMS peak were imposed at various intervals along the spectra and the peaks were calibrated from the nearest side band by finding the relative distances from the TMS peak and taking the average of five to eight determinations for each peak. The values are reported in p.p.m. from the TMS signal taken as zero.

Measurement of pK_a's.—The pK_a's of the heteroyohimbine alkaloids were determined in 67% by volume dimethylformamide in water using a solution of approximately 15 mg. of alkaloid in 30 ml. of solvent. The alkaloids were weighed to the nearest tenth of a milligram and the titrations were made with 0.0509 N aqueous hydrochloric acid. After each addition of 0.050 ml. of HCl, or 0.025 ml. near the calculated pK_a and end point, nitrogen was bubbled through the solution to mix it and remove all carbon dioxide. The pH was then taken with a Beckman pH meter. The pH was then plotted against the milliliters of acid added, the end point was determined at the point of inflection, and the pH found at the point of half neutralization.

Rate Studies.—The kinetics of methiodide formation were measured by following the conductivity of the reaction mixture as a measure of the amount of salt formed. The apparatus consisted of a conventional conductivity cell with black platinized electrodes, an audiooscillator to generate a 1000 c.p.s. current, a Leeds and Northrup wheatstone bridge No. 4760, a condenser to balance the cell capacitance, and an oscilloscope as a galvanometer to measure the balance point. The equipment was assembled so that a null point could be seen on the oscilloscope when the resistance bridge was balanced.

A solution of alkaloid in acetonitrile (spectrograde) made up of 10 mg. of base in 10 ml. of solvent (about. 10^{-3} M) was introduced into the cell. The cell was maintained at 25.0 ± 0.5° by suspending in a constant temperature bath. Nitrogen was originally bubbled through the system, but it was later found that this precaution was not necessary. Methyl iodide (1 ml.) was then added, and at the same time a timer was started. Resistance readings were taken at appropriate intervals until the reaction had practically reached completion, at which time the resistance remained nearly constant.

The alkyl iodide was used in large excess so that the second-order reaction would follow the pseudo-first-order kinetic law

$$-\ln c = kt - \ln c_0$$

where c_0 is the initial concentration of free base and c is the concentration at time t. Since the concentration of alkaloid at time t is equal to the initial concentration less the amount of salt formed c_s, the rate expression can be given by

$$-\ln (c_0 - c_s) = kt - \ln c_0$$

The concentration of salt is proportional to the conductivity of the solution, and since the initial concentration of free base is equal to the final concentration of salt, the rate expression becomes

$$-\ln (1/R_\infty - 1/R_t) = kt - \ln 1/R_\infty$$

where $R\infty$ is the resistance at the end of the reaction and R_t the resistance at time t. If $-\log (1/R_\infty - 1/R_t)$ is plotted against time t, then the slope times 2.3 is equal to the rate constant k, with the y intercept being equal to $-\log 1/R_\infty$.

The measured pseudo-first-order rate constant contains the term for the concentration of alkyl iodide, and thus in any series this concentration must be kept constant if the pseudo-first-order rates are to be compared.

Raunitidine–Isoraunitidine Isomerization.—The general method for carrying out this isomerization was[15]: Two and a half grams of raunitidine in 75 ml. of acetic anhydride and 10 ml. of acetic acid was refluxed under nitrogen for 18 hr. The reaction mixture was decomposed on ice, and the bases extracted with chloroform subsequent to the addition of ammonium hydroxide. After drying over anhydrous magnesium sulfate, the chloroform solution was evaporated down and the residue dissolved in boiling methanol and treated with charcoal. The solution was then evaporated until 2.3 g. of unchanged raunitidine precipitated. The filtrate was evaporated further and 1 g. of oxalic acid was added. From this mixture 190 mg. of isoraunitidine oxalate was isolated, decomposed, and the free base taken into chloroform. After evaporation of the solvent, the residue was recrystallized from methanol giving 100 mg. (4% yield) of isoraunitidine, m.p. 256–258° (reported[15] 258–259°).

When 100 mg. of isoraunitidine was treated under identical conditions, no raunitidine could be isolated, but 97 mg. of isoraunitidine was recovered, which gave 95 mg. of material, m.p. 256–258°, after one recrystallization from ethanol.

When 2.4 g. of raunitidine was refluxed for 4 days and worked up by the above-mentioned method, only 1.15 g. of raunitidine was recovered, and 1 g. of the oxalate salt was obtained. The salt yielded 0.71 g. of crude free base. On washing with 10 ml. of hot methanol, 600 mg. (25% yield) of isoraunitidine, m.p. 255–257°, was obtained for which the infrared spectrum was identical with that of pure isoraunitidine.

3-Dehydroisoraunitidine Perchlorate.—Isoraunitidine was oxidized with mercuric acetate exactly as was done for raunitidine.[15] 3-Dehydroisoraunitidine perchlorate had an infrared spectrum identical with that of 3-dehydroraunitidine perchlorate.

Reduction of 3-Dehydroisoraunitidine Perchlorate.—The perchlorate in methanol was refluxed with sodium borohydride in the same solvent for 2 hr. to give raunitidine, identified by mixture melting point and infrared spectrum.[15]

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