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MITRAGYNA ALKALOIDS: THE STRUCTURE OF STIPULATINE

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In connection with our interest in Mitragyna alkaloids and particularly the unusual 4-methoxy-indole mitragynine[2], we had occasion to examine two batches of M. speciosa, collected six months apart on Luzon in the Phillippines and another collected on Mindanao. Mitragynine was reported[3] found in this species in Borneo but no trace of it was found in our samples. From the bark and leaves of the latter, however, we have succeeded in isolating by the usual procedures[4] rhynocophylline (Ic)[5] and a new alkaloid named stipulatine from its discovery first in the leaves of M. rubrostipulata. We have assigned the structure Ia to this alkaloid as follows.

Stipulatine ($C_{22}H_{28}N_2O_5$, pK_a (50% EtOH) 5.2, $[\alpha]_D^{26.6} = +108° \pm 1°$ ($CHCl_3$), m.p. 238-40°)[6] was shown to contain two methoxyl functions by

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(2) J. B. Hendrickson, Chem. and Ind. 1961, 713.

(3) E. Field, J. Chem. Soc. 119, 887 (1921); H. R. Ing and C. G. Raison, ibid., 1939, 986.

(4) G. M. Badger, J. W. Cook and P. A. Ongley, J. Chem. Soc. 1950, 869.

(5) J. C. Seaton and L. Marion, Can. J. Chem. 35, 1102 (1957); our sample showed infrared spectrum, melting point and mixed melting point identical with those of an authentic sample kindly supplied to us by Dr. Leo Marion.

Zeisel analysis[6] as well as the presence of two three-proton singlets in the proton magnetic resonance spectrum at 6.30 and 6.38 τ. The presence of a C-ethyl group was indicated by a three-proton triplet at 9.12 τ (J=6 cps). The several spectra of stipulatine all showed very strong resemblance to those of rhyncophylline (e.g. infrared peaks at 5.83 μ and 6.15 μ) suggesting the presence of the carbomethoxy-enol-ether and oxindole moieties of the latter. The presence of the oxindole is also indicated by the infrared absorption arising from the NH as well as the presence in the proton magnetic resonance spectrum of a one-proton singlet at 0.84 τ[7] (in $CDCl_3$) which disappears on shaking with a drop of D_2O[8]; this behavior is mirrored in rhyncophylline and various oxindole models. The ultraviolet spectrum (in EtOH) showed two peaks of λ_{max} 222 mμ (22,400) and 292 mμ (2540) with a shoulder at 240 mμ (14,500) which became a distinct peak in acid solution owing to the shift of the lower band from 222 to 218 mμ.

The presence in stipulatine of the ester-enol-ether system of rhyncophylline was demonstrated by their parallel behavior on dilute acid hydrolysis, which converts the latter to the aldehyde rhyncophyllal, IIa[5]. The aldehyde stipulatal, obtained similarly from stipulatine as a chromatographically pure glass[6], was exceedingly similar to rhyncophyllal in properties and spectra, both compounds exhibiting loss of the two methoxyl

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(6) Analyses:

	C	H	N	OCH_3	Mol.wt.*
Stipulatine, calcd. for $C_{20}H_{22}N_2O_3(OCH_3)_2$:	65.98	7.05	7.00	15.50	400
Found :	66.22	7.25	7.19	15.28	400
N-Methystipulatine, calcd. for $C_{23}H_3N_2O_5$:	66.64	7.30	-	-	414
Found:	66.83	7.20	-	-	414
Stipulatal, calcd. for $C_{19}H_{24}N_2O_3$:	69.49	7.37	8.53	-	328
Found :	68.87	7.57	8.85	-	328

*by mass spectrometry

(7) E. Wenkert, J. C. Orr, S. Carratt, J. H. Hansen, B. Wickberg and C. L. Leicht, J. Org. Chem. **27**, 4123 (1962).

(8) H. M. Fales and A. V. Robertson, Tet. Let. 111 (1962).

singlets in the proton magnetic resonance spectrum and appearance of the characteristic aldehyde proton singlet at 0.37 τ . Both compounds yielded a positive Tollens' test.

The nature of the remaining oxygen was revealed as follows: Although the ultraviolet spectrum showed no significant change in alkali, stipulatine afforded a strong ferric chloride color (anhydrous $FeCl_3$ in pyridine), a positive spot test for hindered phenols[9] and a red dye with diazotized sulfanilic acid; as none of these reactions occurred with rhyncophylline, the presence of a phenolic group in stipulatine was inferred. An active hydrogen determination ($LiAlH_4$) supported this with a value of 1.58 active hydrogens per molecule. Treatment of stipulatine with diazomethane led only to quantitative recovery of the alkaloid while acetylation with refluxing acetic anhydride and sodium acetate afforded the monoacetyl derivative Ic (m.p. 170-71°) characterized by infrared and proton magnetic resonance spectra (loss of the oxindole NH and appearance of a three-proton singlet at 7.33 τ). With excess dimethyl sulfate the alkaloid was converted to N-methylstipulatine, Ib (m.p. 77-79°)[6], the placement of the methyl group deduced as above from the proton magnetic resonance; Ib showed OH absorption in the infrared at 2.8 μ. Attempts to acetylate Ib afforded a dark oil which showed a new infrared peak at 5.6 μ but which could not be purified without substantial reversion to Ib. All the above derivatives gave positive ferric chloride tests.

The position of the oxygen on the aromatic ring was deduced from a careful examination of the aromatic region of the proton magnetic resonance spectra of stipulatine and its derivatives and by consideration of the singular lack of reactivity of this phenolic group.

The aromatic region of the spectrum of stipulatine showed seven peaks

(9) G. H. Stillson, D. W. Sawyer and C. K. Hunt, J. Am. Chem. Soc. 67, 303 (1945).

with a total area equal to four protons. One of the seven peaks (2.77τ, area = one proton) could be assigned to the proton on C_{17} of the enol ether system[10]. The six remaining peaks were two "triplets" centered at 2.55τ (one proton) and 3.53τ (two protons). At 100 mc the lower "triplet" remains as such but the higher one is resolved into two doublets (3.42τ, J = 7.4 cps; 3.54τ, J = 7.7 cps.). While the aromatic region of Ib at 60 mc. is qualitatively the same as that of Ia at 100 mc ((triplet": 2.90τ; 2 doublets: 3.10τ, J = 8 cps; 3.65τ, J = 7.5 cps), the same region of Ic is instructively different, consisting again of a "triplet" (2.78τ) and two doublets (3.20τ, J = 8.5 cps; 2.26τ, J = 8.0 cps), but one of the doublets has been shifted downfield by about 50 cps!

These spectra are best explained by a 1,2,3-aromatic proton pattern in which two of the protons are shifted upfield by the shielding effect of an ortho oxygen or nitrogen. These two upfield protons split the third proton almost equivalently producing a "triplet" signal. The placement of the hydroxyl at C_9 rather than at C_{12} is based on the fact that upon N-acetylation one proton is deshielded considerably by the acetyl group and so must be a proton ortho to the nitrogen at C_{12}. Furthermore, the aromatic regions of the indole alkaloids, aspidospermine[11] and cylindro-carpine[11], which can be used as models for the C_{12} hydroxyl group[7], donot resemble that of stipulatine and its derivatives. Finally, the spectrum of 1-methyl-4-methoxy-oxindole[12] is very similar to that of stipulatine, showing the separated aromatic triplets at 2.75τ (one proton) and 3.48τ (two protons), while 1-methyl-7-methoxy-oxindole[13] affords a very different spectrum featuring

(10) E. Wenkert, B. Wickberg and C. Leicht, Tet. Let. 822 (1961).

(11) C. Djerassi, A.A.P. G. Archer, T. George, B. Gilbert, J. N. Schoolery and L. F. Johnson, Exper. 16, 532 (1960); C. Djerassi, B. Gilbert, J. N. Schoolery, L. F. Johnson and K. Biemann, Exper. 17, 162 (1961).

(12) J. D. Loudon and J. Ogg, J. Chem. Soc. 1955, 739.

(13) Kindly supplied us by Dr. J. D. Loudon, Glasgow.

only a single multiplet at 2.18 τ. Alkaloids with 5- and 6-methoxy-oxindole and indole moieties also exhibit very different spectra[7,11,14]. Finally, the unreactivity of this phenol tends to support its location at C_{12} adjacent to the hindering ortho quaternary carbon (cf., o-t-butylphenol).

These data allow the formulation of stipulatine as Ia, which is confirmed by the mass spectra of stipulatine and its derivatives. A recent study of the mass spectra of oxindole alkaloids[15] shows a dominant fragment from the right-hand half of the molecule, formulated as  and thus occurring in rhynocophylline as a strong peak of m/e 239; this is accompanied by peaks at m/e 224 (loss of methyl), 210 (loss of ethyl), and 208 (loss of methoxyl). As expected from Ia, the mass spectra of stipulatine and N-methyl-stipulatine show the identical set of fragments, while the dominant peak from stipulatal is m/e 167 which corresponds to the m/e 239 peak of the others. The peaks assigned[15] to the oxindole portion of rhyncophylline and the other oxindole alkaloids are found at m/e 130, 144-6, and 159. Stipulatine and stipulatal show a peak at m/e 146 (addition of one oxygen to m/e 130) while N-methyl-stipulatine has in this region only the expected m/e 160 and 174-6 from addition of oxygen and N-methyl to the parent oxindole spectrum.

It is characteristic of these oxindole bases to equilibrate between normal and isoforms involving epimerization at C_3 and C_7 in hot acid or base[16, 17]; while in the other known cases substantial amounts of each isomer are formed in this equilibration, this is not true of stipulatine, which formed an isomer

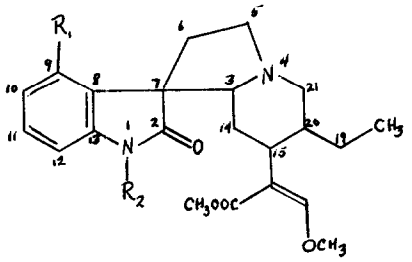
(14) M. Gorman, N. Neuss and K. Biemann, J. Am. Chem. Soc. 84, 1058 (1962).

(15) B. Gilbert, J. A. Brissolese, N. Finch, W. L. Taylor, H. Budzikiewicz, J. M. Wilson and Carl Djerassi, J. Am. Chem. Soc. in press; we are grateful to Dr. Taylor for a copy prior to publication.

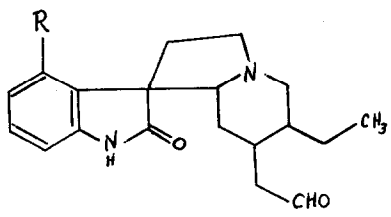
(16) J. C. Seaton, M. D. Nair, D. E. Edwards and L. Marion, Can. J. Chem. 38, 1035 (1960).

(17) N. Finch and W. I. Taylor, J. Am. Chem. Soc. 84, 3871 (1962).

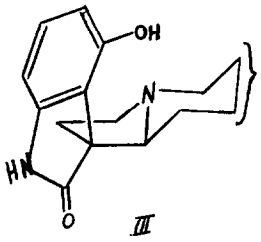
only in minute yield as evidenced by thinlayer chromatography. Comparison of stipulatine with the other oxindoles[17] suggest it has the iso-configuration, III, inferred from the large positive rotation and the grester R_f value of stipulatine over its isomer. Models make it clear, furthermore, that in the iso-configuration (III) there is compelling geometry for hydrogen-bonding of the phenolic-OH to N_b. Such a hydrogen-bond would account for several observations, viz., (1) the insensitivity of the ultraviolet spectrum to dilute hydroxide, (2) the lack of any well-defined peak assignable to the phenolic hydroxyl in the proton magnetic resonance spectrum, and (3) the apparent unbalancing of the normal/iso equilibrium in favor of the H-bond- stabilized iso-form inferred from the very small yields of isomer in acid or base equilibration.



Ia, R_1 = OH, R_2 = H
b, R_1 = OH, R_2 = CH_3
c, R_1 = OH, R_2 = $COCH_3$
d, R_1 = H, R_2 = H



IIa, R = H
b, R = OH



III

Stipulatine may thus be added to the very small group of natural 4-oxygenated indole derivatives and is the first such oxindole to be found

in nature; pharmacological studies are in progress.

We should like to express our gratitude to Dr. Murle Klohs of Riker Laboratories for various samples of Mitragyna plant material, to Mr. N.S. Bhacca of Varian Associates for proton magnetic resonance spectra at 100 mc, to Professor K. Biemann of M.I.T. for mass spectra, and to Drs. W. D. Ollis and W. I. Taylor for helpful discussions of the problem. Finally it is a pleasure to acknowledge the support of the National Science Foundation through grant No. G19794.