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STUDIES IN THE ALKALOIDS OF RAUWOLFIA CAFFRA SONDER

Part III. The Structure of Raucaffricine

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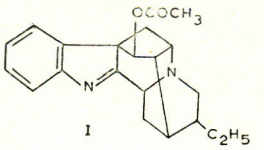
Abstract. Raucaffricine has been proved to be D(+)–galactoside of vomelenine at C-21 oxygen mainly by its spectral studies and that of its acid hydrolytic constituents. It has further been substantiated by partial enzymic synthesis.

The isolation of raucaffricine alongwith ajmaline, ajmalicine, perakine, raucaffriline and raucaffridine from *Rauwolfia caffra* Sonder was reported[1] in 1964. The preliminary pharmacological reports for this alkaloid indicated an hypotensive activity one-and-half-times as potent as reserpine. In view of this fact, it was considered of interest to examine its structure. Raucaffricine was suggested[2] earlier to belong to the group of compounds derived from N_α–dimethyl –△′-deoxyajmaline–17–*O*–acetate (I), on the basis of studies in the sodium borohydride reduction of raucaffricine and perakine.



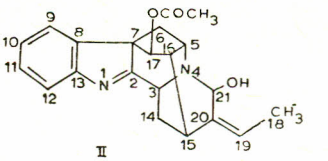
I

In a preliminary communication last year[3] the structure of raucaffricine was proposed mainly on the basis of spectral studies, investigations of its hydrolytic products and its partial synthesis. The elucidation of its chemical structure and stereochemistry has been elaborated in the present communication.

In part I of this series,[1] the molecular formula $C_{26}H_{32}O_8N_2.\frac{1}{2}H_2O$ was proposed for raucaffricine $C_{34}H_{40}O_{12}N_2$ for its tetracetate, $C_{54}H_{48}O_{12}N_2$ for the tetrabenzoate and $C_{26}H_{32}O_8N_2.CH_3$ for the methiodide. It was also shown that raucaffricine which crystallises from ethyl acetate–methanol in hexagonal plates, m.p. 220°C, $[\alpha]_D^{30} + 14.5$ (ethanol) had four hydroxyl groups, one *O*-acetyl group and no methoxyl group, thus accounting for 6 out of its 8 oxygen atoms. It reduces Fehling's and ammoniacal silver nitrate solutions, though neither the IR spectrum of the base nor the NMR spectrum of the tetraacetyl derivative shows the presence of an aldehyde group: oxime and phenylhydrazone are also not obtained. Further, the NMR spectrum of the tetraacetyl derivative did not show the presence of >NH or —OH groups.

The alkaloid, therefore, appeared to be a glycoside and was hydrolyzed by heating at 100°C with 1N HCl for ½ hr. This hydrolysis yielded two components, one of which was identified as D(+)–galactose through paper chromatography as detailed below. Hydrochloric acid was removed from the hydrolysate and the residue dried. It was taken up in water and run on Whatman paper No. 1. Development by benzene–n-butanol–pyridine–water (1:5:3:3)[4] and spraying of the chromatogram with a saturated solution of aniline oxalte revealed the sugar as D(+)-galactose which was confirmed by preparation of its osazone.

As regards the aglycone, the UV spectrum of raucaffricine exhibits absorption in the regions known for indolenine alkaloids λ_{max} 219.5, 258 nm λ_{min} 236 nm.[1] It was proposed earlier that the easy conversion of raucaffricine to indole bases under mild alkaline conditions, similar to such conversion of perakine,[5] suggests that it is an indolenine derivative. Thus we recorded the NMR spectrum (60 MHz) in pyridine and observed a close similarity to that of vomilenine (II).[6]



II

Attention is specially drawn to the peaks for the ethylidine group (1.56δ, 3H,d, *J* 6.7 Hz; 5.98δ, 1H,q, *J* 7 Hz) the acetyl group (2.23δ, 3H,s), the single hydrogens at C-15 (2.80δ, 1H,m), C-5 (3.33δ, 1H,q) and at C-3 (4.31δ, 1H). The peak centred at 5.00δ corresponds to the peak in the vomilenine spectrum[6] at exactly the same position. A noteworthy difference in the two spectra is for the anomeric hydrogen atom which appears as a broad doublet at 5.42δ.[7]

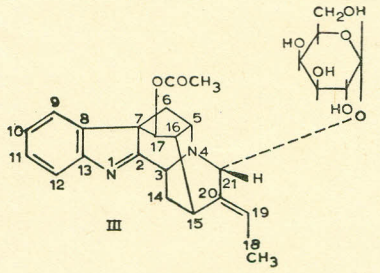
For confirming that the aglycone was indeed vomilenine, the hydrochloride of the base that was obtained from an hydrolysis experiment with 1N HCl was

compared with that obtained from a known solution of vomilenine similarly refluxed in the same solvent. Both showed identical IR and UV spectra and gave the same R_f values on TLC. The free bases were again isolated therefrom and compared by IR and UV spectra and TLC which were found identical. The UV spectrum showed typical absorptions for the indole structure indicating that the base had changed completely from the indolenine to the indole type on heating with the acid as may be expected.[5]

For finding out the nature of the linkage at the anomeric carbon an attempt was made to hydrolyse the alkaloid with sweet almond emulsion which contains β-galactosidase, but, to a lesser extent α-galactosidase. As it is not hydrolysed to any appreciable extent it is possibly an α-galactoside.

In order to establish that raucaffricine was indeed an α-galactoside of vomilenine its enzymic partial synthesis (vomilenine has not been synthesized so far) was attempted with the latter and D(+)-galactose (large excess) in presence of fresh brewer's yeast. After intermittent shaking of the mixture for 10 days at 25°C, TLC (butane–acetic acid–water, 4:1:1) revealed the presence of raucaffricine (compared with a known sample run alongside).

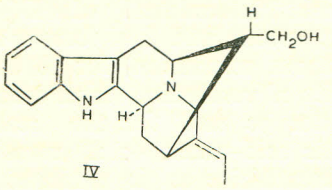
On the basis of the above findings structure III was proposed for raucaffricine and the molecular formula



III

for the base was modified to $C_{27}H_{32}O_8N_2 . \frac{1}{2}H_2O$ (requires: C, 62.2; H, 6.33; O, 26.11; N, 5.38; active H, 1.16; C—CH_3, 5.77; acetyl value, 8.25% and mol wt. 521. Found after drying at 100°C over P_2O_5 *in vacuo*: C, 61.84; H, 6.30; O, 26.04; N, 5.71; active H, 1.2; acetyl value 9.09% and mol. wt. 506) and thus the tetraacetate becomes $C_{35}H_{40}O_{12}N_2$. Found: C, 61.15; H, 5.91; O, 28.33; N, 4.56; acetyl value 31.88 and mol. wt. 642; $C_{35}H_{40}O_{12}N_2$ requires: C, 61.75; H, 5.92; O, 28.21; N, 4.12, acctyl value, 31.61% and mol. wt. 681. Found: C, 70.66; H,5.13; O, 21.09; N, 3.37%; The tetrabenzoate $C_{55}H_{48}O_{12}$ requires: C, 71.10; H, 5.21; O, 20.67; N, 3.02%. Found: C, 50.49; H,5.55; N, 3.97; I, 19.60 and N-CH_3, 2.60%. The methiodide $C_{27}H_{32}O_8N_2 . CH_3I$ requires; C, 50.48; H, 5.49; N, 4.36; I, 19.67 and N-CH_3, 2.34%.

Molecular models of raucaffricine also indicate that the preferred glycosidic linkage at the anomeric C atom is α, and that the C–21 oxygen of the vomilenine moiety is axial(III). (However for vomilenine, as the free base, being a carbinolamine, equilibration at that junction is possible. Similar steric considerations imply that the geometry of the C–18 methyl attached to the ethylidine group is possibly that of the sarpagine alkaloids(IV).[8]



IV

Experimental

Enzymic Hydrolysis of Raucaffricine with Sweet Almond Emulsin. Raucaffricine (20 mg) was dissolved in distilled water (1 ml) and sweet almond emulsin (0.1 ml) prepared by the method given in ref. 9), and the reaction mixture was heated on a water-bath maintained at 43°C with occasional shaking. Subsequently the quantity of enzyme was gradually increased to 0.7 ml. The experiment was run for ca. 2 week during which it was heated at 43°C for 26 hr. Raucaffricine was still present as shown by thin layer chromatography silica gel G plate (solvent system used: butanol–acetic acid–water, 80:20:20).

Hydrolysis of Raucaffricine by Water. The base (5 mg) was heated with distilled water (1 ml) at 100°C for 5 hr when it went into solution gradually. Partial hydrolysis was indicated on TLC plate in Partridge[10] solvent system.

Acid Hydrolysis of Raucaffricine. Raucaffricine (20 mg) was dissolved in 5 ml HCl (1N) and heated to boiling for 30 min. It was freed of the solvent *in vacuo* and the residue partitioned in absolute alcohol. The absolute alcohol insoluble fraction (7 mg) was examined through paper chromatography along with D-glucose, D-galactose and mannose on Whatman paper No. 1 developed by benzene–n-butanol–pyridine–water (1:5:3:3). The chromatogram was sprayed with a saturated solution of aniline oxalate when it indicated D-galactose ($R_f = 0.25$). The absolute alcohol–insoluble fraction formed osazonewith phenylhydrazine hydrochloride which was identical with the osazone of an authentic sample of D(+)–galactose.

The absolute alcohol-soluble fraction was freed of the solvent *in vacuo* and the residue thus obtained was taken up in water (ca. 1 ml). It was then basified with 10% NH_4OH and the liberated base was extracted from ethyl acetate. The ethyl acetate solution was washed with water, dried (Na_2SO_4) and freed of the solvent *in vacuo*. The basic residue (13 mg) could not be crystallised. Its IR spectrum indicated—OH absorption peak at 3400 cm^{-1} and no carbonyl absorption. Its UV absorption spectrum was of a typical indole derivative (λ_{max} 228, 285 nm and a shoulder at 292 nm). The base could not be further characterised due to paucity of the material and was identified through TLC over a silica gel (G)PF/254 plate alongwith authentic samples of raucaffricine, vomilenine and perakine as such, and vomilenine worked up after refluxing in 1N HCl under identical conditions.

The R_f values of raucaffricine hydrolysate was equal to that of vomilenine hydrolysate in butanol–acetic acid–water, 4:1:1.

Partial Synthesis of Raucaffricine. Vomilenine (1 mg) was shaked intermittently with D(+)–galactose (10 mg, large excess) and fresh brewer's yeast (two drops of aqueous suspension) for 10 days at 25°C. Due to paucity of vomilenine larger amounts of the base could not be used for the reaction. TLC (butanol–acetic acid–water, 4:1:1) revealed the formation of raucaffricine as it exhibited the same R_f with an authentic sample run alongside.

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