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ALKALOIDS FROM *CROTON PLUMIERI*

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Abstract—*Croton plumieri* Urb. has been shown to contain the alkaloids crotonosine, L-*N*-methylcrotonosine, salutaridine and 8,14-dihydrosalutaridine. A comparison is made with the alkaloid contents of *C. linearis* Jacq. and *C. flavens* L. which are now believed to be the parents of the hybrid *C. plumieri*.

IN CERTAIN parts of the Port Henderson area, Jamaica, the *Croton* population consists mainly of *C. linearis* Jacq., *C. flavens* L. and *C. plumieri* Urb. *C. linearis* is dioecious with linear-oblong leaves, while *C. flavens* is monoecious with leaves ovate-lanceolate to ovate, being mainly entire, but sometimes serrate.[1] *C. plumieri* shows characteristics intermediate between the two, and it seemed possible that *C. plumieri* could be a natural hybrid between *C. linearis* and *C. flavens*.[2] This postulate has received strong support from artificial hybridization experiments undertaken by Dr. G. Chapman at the University of the West Indies.[3] The artificial hybrids were produced from seeds of open pollinated flowers of a female *C. linearis* plant growing in close proximity to a flowering *C. flavens* species. All the hybrids so produced were monoecious, and were indistinguishable morphologically from the natural hybrids examined.

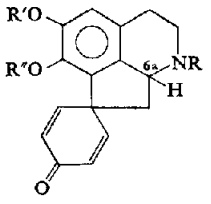
It is planned to undertake a chemical survey of these hybrids in conjunction with their genetic analysis in a manner analogous to the work of Smith on certain *Nicotiana* species.[4] At this stage we report the isolation work on plant material obtained from one morphologically identical stand of the naturally occurring hybrid.

Earlier work on *C. linearis* has shown that this plant contains at least eight chloroform soluble alkaloids. The major alkaloid is crotonosine (Ia). This is accompanied by pronuciferine (Ib), L-*N*-methylcrotonosine (Ic), linearisine (IIa),[5] base E (IIb),[6] jacularine (IIc),[7] 8,14-dihydrosalutaridine (IIIa) and 8,14-dihydronorsalutaridine (IIIb).[8] *C. flavens* growing in the Port Henderson area was shown to have a different alkaloid content for the serrate leaf variety than for the entire leaf type.[9] Norsinoacutine (Va) and flavinine (VIa) were isolated from the serrate leaf type, while norsinoacutine (Va), sinoacutine (Vb) and flavinantine (VIb) were found in the entire leaf type. This difference was not observed, however,

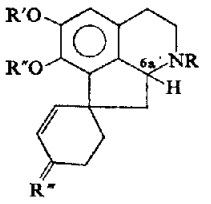
[1] W. FAWCETT and A. B. RENDLE, *Flora of Jamaica*, Vol. 4, p. 279, The British Museum, London (1920).
[2] D. ADAMS and G. CHAPMAN, unpublished observations.
[3] G. CHAPMAN, personal communications.
[4] H. H. SMITH, *Am. Nat.* **99**, 73 (1965).
[5] L. J. HAYNES, K. L. STUART, D. H. R. BARTON and G. W. KIRBY, *J. Chem. Soc.* (C) 1676 (1966).
[6] L. J. HAYNES, G. E. M. HUSBANDS and K. L. STUART, *J. Chem. Soc.* (C) 1680 (1966).
[7] K. L. STUART, L. J. HAYNES, M. BARRETT and G. E. M. HUSBANDS, *Tetrahedron Letters* 4473 (1968).
[8] L. J. HAYNES, G. E. M. HUSBANDS and K. L. STUART, *J. Chem. Soc.* (C) 951 (1968).
[9] C. CHAMBERS and K. L. STUART, *Chem. Commun.* 328 (1968).

for cultivated plants in the Mona area. At Mona, both leaf types were shown to contain norsinoacutine, sinoacutine and flavinantine.[10] This could either be due to seasonal changes or a difference in the soil types.

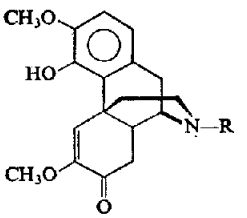
C. plumieri collected from Port Henderson was shown to contain crotonosine (Ia), L-*N*-methylcrotonosine (Ic), 8,14-dihydrosalutaridine (IIIa) and salutaridine (IV) as the



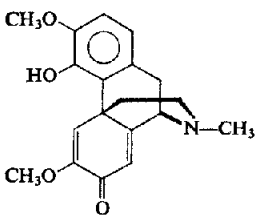
	R	R′	R″	6a Configuration
(I) (a) Crotonosine	H	H	CH_3	R
(b) Pronuciferine	CH_3	CH_3	CH_3	R
(c) L-*N*-Methylcrotonosine	CH_3	H	CH_3	S
(d) Crotsparine	H	CH_3	H	S



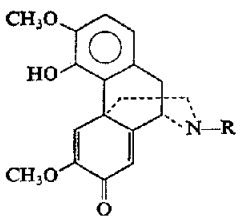
	R	R′	R″	R‴	6a Configuration
(II) (a) Linearisine	CH_3	H	CH_3	O	S
(b) Base E	CH_3	CH_3	H	H, OH	R
(c) Jacularine	H	CH_3	H	O	R



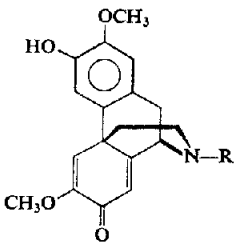
(III) (a) 8,14-Dihydrosalutaridine, R = CH_3
(b) 8,14-Dihydronorsalutaridine, R = H



(IV) Salutaridine



(V) (a) Norsinoacutine, R = H
(b) Sinoacutine, R = CH_3



(VI) (a) Flavinine, R = H
(b) Flavinantine, R = CH_3

major chloroform soluble alkaloids. There was some TLC evidence which suggested that 8,14-dihydronorsalutaridine and linearisine could also be present.

C. plumieri, like *C. linearis*, biosynthesises both proaporphine and morphinandienone type alkaloids. It has been postulated that 8,14-dihydrosalutaridine (IIIa) is formed from salutaridine (IV),[8] and although salutaridine has not yet been isolated from *C. linearis*, it could, however, be present in such small quantities that radioisotopic dilution methods, similar to those used by Barton, Battersby and their colleagues on *Papaver somniferum* Noordster plants would be required to detect it.[11] The isolation of salutaridine along with 8,14-dihydrosalutaridine from *C. plumieri* is, however, heartening support for the view that these compounds are biosynthetically related. Appropriate radioisotopic experiments have been initiated to test their relationship, notwithstanding the findings by Barton and co-workers that sinoacutine (Vb) was shown not to be the precursor of isosinomenine (IIIa enantiomer) in *Sinomenium acutum* plants.[12]

Due to the slow growing nature of the hybrid *C. plumieri* (approximately four years to flowering), it would be difficult to carry out a hybridization study similar to that undertaken by Danos,[13] Tetenyi and Vagujfalvi[14] on *P. somniferum*. Danos found that the qualitative characteristics of the parents and the typical alkaloid composition were cumulated in the hybrid offspring in the first filial generation. Our results show that in the case of the natural hybrids examined, the alkaloid composition was not cumulative. It should, however, be borne in mind that salutaridine (IV) is the enantiomer of the *C. flavens* alkaloid, sinoacutine (Vb), and recently Bhakuni found that *C. sparsiflorus* Morang produces crotsparine (Id) growing around Calcutta (East India) but yields the enantiomer in North-Central India.[15]

EXPERIMENTAL

Extraction of Chloroform Soluble Bases from Croton plumieri

Stem and leaves of *C. plumieri* from Port Henderson were chopped and then dried at 60° for 2 days in an infrared oven, then powdered in a hammer mill. The dried plant material (662 g) was extracted with 14 l. of 2% tartaric acid solution. The extract was concentrated in a cyclic evaporator to 1 l. After adjusting to pH 9 with 25% ammonia it was continuously extracted with $CHCl_3$ for 8 hr. Removal of the $CHCl_3$ yielded 8·87 g of a crude alkaloid mixture, which represents a 1·34 per cent yield based on the dried weight of plant material.

Separation of Alkaloids

The crude alkaloid mixture (6 g) was separated in a 120 tube semi-automatic countercurrent apparatus using $CHCl_3$ as the stationary phase (40 ml tubes) and 0·2 N sodium acetate-acetic acid buffer (pH 4·76) as the moving phase. The partition ratio was 1·35 at this pH. 60 transfers were effected. The separation of bases was monitored with the aid of TLC (silica coated plates developed in $CHCl_3$-MeOH, 1:1, and compounds made visible with iodine vapour). The countercurrent tubes were grouped into 6 fractions. Alkaloid material was transferred into the organic phase by adding ammonia and effecting a complete transfer by using additional quantities of $CHCl_3$.

Tubes 4–6. Fraction 1. Purification of this material on an alumina column in methanol yielded 20 mg of material which had identical physical properties (TLC, optical rotation, i.r.) to salutaridine. Mixed m.p. with an authentic sample of salutaridine showed no depression.

Tubes 8–10. Fraction 2. Salutaridine (52 mg) was also obtained from this fraction after purification on an alumina column as in the case of fraction 1, and its identity verified as above.

[10] K. L. STUART, unpublished results.
[11] D. H. R. BARTON, G. W. KIRBY, W. STEGLICH, G. M. THOMAS, A. R. BATTERSBY, T. A. DOBSON and A. RAMUZ, *J. Chem. Soc.* 2423 (1965).
[12] D. H. R. BARTON, A. J. KIRBY and G. W. KIRBY, *J. Chem. Soc.* (C) 929 (1968).
[13] B. DANOS, *Pharmazie* **20**, 727 (1965); *Chem. Abs.* **64**, 8654f (1966).
[14] P. TETENYI and D. VAGUJFALVI, *Pharmazie* **20**, 731 (1965); *Chem. Abs.* **64**, 10088e (1966).
[15] D. S. BHAKUNI and M. M. DHAR, *Experientia* **24**, 1026 (1968).

Tubes 18–26. Fraction 3. This fraction, which was shown to be a mixture of at least two alkaloids, was further separated by preparative TLC, but no crystalline material was obtained. These minor alkaloids have not been identified even after several TLC comparisons with known alkaloids from *C. linearis* and *C. flavens*.

Tubes 30–36. Fraction 4. This fraction (990 mg) showed the presence of three alkaloids on TLC. 490 mg of this mixture was further separated by the Bush and Densen extraction procedure.[16] 0·2N sodium acetate-acetic acid buffer (pH 4·99) was used along with $CHCl_3$ and separation effected with the aid of 36 separating funnels and using 40 ml of each phase for each operation. 12 fractions were obtained and fraction 8 gave a single spot on TLC, $[\alpha]_D^{20} - 76°$ (*c* 0·5 in MeOH); (lit. $[\alpha]_D^{15} - 69·1°$ in MeOH)[8] and the i.r. spectrum was that of 8,14-dihydrosalutaridine. TLC examination of the other fractions indicated that linearisine and 8,14-dihydronorsalutaridine could also be present, but conclusive proof of the presence of these compounds is still needed.

Tubes 40–50. Fraction 5. Material from this fraction (75 mg) gave one spot on TLC in several solvent systems. The addition of a few drops of a methanol-acetone mixture resulted in the formation of crystals. Recrystallization from ethanol gave anisotropic plates (53 mg), m.p. 210–213°, $[\alpha]_D^{15} - 105°$ (*c* 0·99 in MeOH) representing a 0·008 per cent yield based on the dried weight of the plant. The i.r. spectrum in nujol gave an identical spectrum to that of L-*N*-methylcrotonosine.

Tubes 53–60. Fraction 6. This fraction (203 mg) also contained one major compound, but because of its brown colour, further purification was necessary. This was effected on an alumina column packed in methanol. The pale orange coloured eluent yielded, on evaporation, a compound, $[\alpha]_D^{20} + 187·7°$ (*c* 1·08 in MeOH) indistinguishable from crotosonine (i.r. and TLC).

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[16] M. T. BUSH and P. M. DENSEN, *Anal. Chem.* **20**, 121 (1948).