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Alkaloids of Tylophora I: Isolation of Six New Alkaloids

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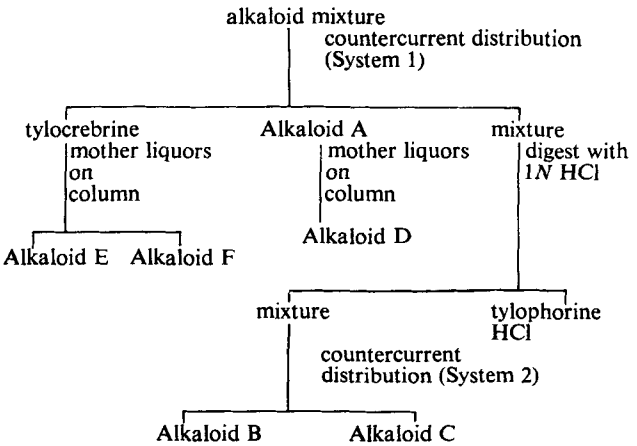
Abstract □ *Tylophora crebriflora* (N. O. Asclepiadaceae) is a slender vine found chiefly in northeastern Australia. In a detailed examination of the plant, six alkaloids have been isolated which have not previously been shown to be present in this genus. The methods for their isolation and their physical characteristics are described.

Keyphrases □ *Tylophora crebriflora*—separation, isolation, physical properties, six alkaloids □ IR spectrophotometry—structure, identification □ UV spectrophotometry—structure, identification

In a series of papers during the 1950's, Govindachari *et al.* (1–5) described the isolation, structure, and synthesis of two alkaloids, tylophorine and tylophorinine, present in the Indian plant *Tylophora indica*. These alkaloids are built up of a dibenzo[*f,h*]-pyrrolo[1,2*b*]-isoquinoline skeleton. From a related Australian plant, *Tylophora crebriflora*, Gellert *et al.* (6) described the isolation of a third member named tylocrebrine, together with a minor amount of tylophorine. The two were shown to be isomeric, differing in the arrangement of the methoxyl groups. During routine screening by the Cancer Chemotherapy National Service Center (CCNSC), it was observed that tylocrebrine showed significant antileukemic activity. At the request of CCNSC to provide tylocrebrine for possible clinical trials, these studies were initiated.

EXPERIMENTAL

The dried plant, *Tylophora crebriflora*, was obtained from Australia.[1] The total alkaloid fraction could be readily isolated by the following steps: (*a*) extraction with 1% methanolic acetic acid; (*b*) concentration; (*c*) partition between ethyl acetate and 0.2 *N* HCl (aq.); and (*d*) extraction of the aqueous layer at pH 9–10 with chloroform. The crude mixture of alkaloids represented a yield of approximately 0.15%.



Scheme I—Separation of the alkaloids of Tylophora crebriflora. *System 1: 3% aqueous acetic acid–chloroform–ethyl acetate (10:7:3) System 2: 3% aqueous acetic acid–chloroform–*n*-butanol (5:4:1)*

The mixture was separated into its components by the use of countercurrent distribution and chromatography on a commercial adsorbent,[2] as indicated in Scheme I. In addition to the two known members, tylocrebrine and tylophorine, the extracts yielded six new alkaloids.

Tylocrebrine and Alkaloid A are the major components, each being present to the extent of about 40% of the total. Next in abundance are tylophorine and Alkaloids B and C, which account for approximately 4–5% each. The rest is made up of the other three members, Alkaloids D, E, and F.

The analytical data and physical properties of the new members are shown in Tables I and II. In general, Alkaloids A–E show

[1] The plant material used in this study was collected, identified, and supplied by the Department of Forestry of Queensland, Brisbane, Queensland, Australia, in 1964. (A voucher specimen was preserved at Chas. Pfizer & Co., Inc., Maywood, N. J.)

[2] Florisil, Floridin Co., Pittsburgh, Pa.

Table I—Characteristics of the Tylophora Alkaloids

Property	Alkaloid A	Alkaloid B	Alkaloid C
1. Melting point	212–214°	222–224°	223–225°
2. Formula	$C_{24}H_{27}NO_5$	$C_{23}H_{25}NO_4$	$C_{23}H_{25}NO_5$
3. Analysis	Calcd. / Found	Calcd. / Found	Calcd. / Found
	C, 70.40 / C, 70.26	C, 72.80 / C, 72.41	C, 69.85 / C, 69.42
	H, 6.65 / H, 6.62	H, 6.64 / H, 6.69	H, 6.37 / H, 6.44
	N, 3.42 / N, 3.43	N, 3.69 / N, 3.61	N, 3.54 / N, 3.55
	OMe, 30.31 / OMe, 30.16	OMe, 24.53 / OMe, 24.49	OMe, 23.07 / OMe, 23.54
4. UV spectrum	$\lambda_{max.}$ / log ϵ	$\lambda_{max.}$ / log ϵ	$\lambda_{max.}$ / log ϵ
	262 / 4.837	262 / 4.818	262 / 4.777
	285(sh) / 4.425	284(sh) / 4.386	265(sh) / 4.359
	305(sh) / 4.028	302(sh) / 4.057	303(sh) / 4.143
5. Rotation $[\alpha]_D^{25}$ (C, 1 in chloroform)	−32	−63	34
6. R_f: Citric acid–formamide–chloroform[a]	0.2–0.3	0–0.1	0–0.1
Formamide–chloroform	0.65–0.75	0.5–0.6	0.3–0.4

[a] Whatman No. 1 sheets immersed in 5% citric acid, dried, moistened before use with 30% formamide in methanol, and developed with chloroform saturated with formamide.

Table II—Characteristics of the Tylophora Alkaloids

Property	Alkaloid D	Alkaloid E	Alkaloid F
1. Melting point	186–188°	198–200°	137–138°
2. Formula	$C_{25}H_{29}NO_6$	$C_{25}H_{29}NO_5$	$C_{24}H_{29}NO_4$
3. Analysis	Calcd. / Found	Calcd. / Found	Calcd. / Found
	C, 68.32 / C, 68.36	C, 70.90 / C, 70.67	C, 72.88 / C, 72.74
	H, 6.65 / H, 6.80	H, 6.80 / H, 6.80	H, 7.39 / H, 7.37
	N, 3.19 / N, 3.19	N, 3.31 / N, 3.88	N, 3.54 / N, 3.52
	OMe, 35.30 / OMe, 34.95	OMe, 36.64 / OMe, 36.21	OMe, 31.39 / OMe, 31.40
4. UV spectrum	$\lambda_{max.}$ / log ϵ	$\lambda_{max.}$ / log ϵ	$\lambda_{max.}$ / log ϵ
	262 / 4.843	263 / 4.878	240(sh) / 4.198
	282(sh) / 4.410		288 / 4.001
5. Rotation $[\alpha]_D^{25}$	−16.5	−69	−42.5
6. R_f: Citric acid–formamide–chloroform	0.4–0.5	0.7–0.8	0.7–0.8

characteristics very similar to those of tylocrebrine, thus showing similarity in chemical structure. Alkaloid F has somewhat different spectral properties, and the exact significance of these results will be discussed in a subsequent paper.

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