jection of the phlogistic agent. This was done to offset any possible error caused by the difference in the size of the animals used. The standard error of the mean (*SEM*) was calculated according to the formula given by Burn *et al.* (10):

$$s\bar{y} = \sqrt{\frac{S(y - \bar{y})^2}{N(N-1)}} \qquad \text{(Eq. 1)}$$

where $s\bar{y}$ = *SEM*, $\bar{y}$ = mean value, y = any single value, N = total number of animals used in the assay, and S = sum.

The method and techniques developed for the anti-inflammatory screening carried out in this investigation proved to be quite satisfactory. The method was simple and quick, and the apparatus was sufficiently sensitive for the determination of therapeutic levels of antiedema activity. Results with the hydrocortisone acetate and indomethacin, the control compounds, compared favorably with the results of others using antiedema assay procedures (2). Carrageenin seemed to possess a distinct advantage as a phlogistic agent because it produced an edema effectively controlled by the single, oral, nontoxic doses of the known anti-inflammatory agents used in the studies.

REFERENCES

(1) T. Y. Shen, T. B. Windholz, A. Rosegay, B. E. Witzel, A. N. Wilson, J. D. Willett, W. J. Holtz, R. L. Ellis, A. R. Matzuk, S. Lucas, C. H. Stammer, F. W. Holly, L. H. Sarett, E. A. Risley, G. W. Nuss, and C. A. Winter, *J. Amer. Chem. Soc.*, **85,** 488(1963).

(2) C. A. Winter, E. A. Risley, and G. Nuss, *J. Pharmacol. Exp. Ther.*, **141,** 369(1963).

(3) C. V. Winder, J. Wax, L. Scotti, R. A. Scherrer, E. M. Jones, and F. W. Short, *ibid.*, **138,** 405(1962).

(4) C. V. Winder, J. Wax, B. Serrano, E. M. Jones, and M. McPhee, *Arthritis Rheum.*, **6,** 36(1963).

(5) K. J. Rorig and H. A. Wagner, U. S. pat. 3,149,109 (Sept. 15, 1964).

(6) B. H. Chase and W. T. Weller, *J. Pharm. Pharmacol.*, **16,** 163(1964).

(7) W. B. Deichman and T. J. LeBlanc, *J. Ind. Hyg. Toxicol.*, **25,** 415(1943).

(8) M. W. Jordin and W. D. Easterly, Jr., *J. Pharm. Sci.*, **54,** 1190(1965).

(9) C. A. Winter, E. A. Risley, and G. Nuss, *Proc. Soc. Exp. Biol. Med.*, **111,** 544(1962).

(10) J. H. Burn, D. J. Finney, and L. G. Goodwin, "Biological Standardization," 2nd ed., Oxford University Press, London, England, 1960, p. 33.

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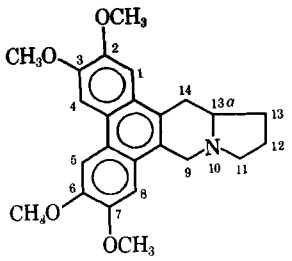
Alkaloids of Tylophora III: New Alkaloids of *Tylophora indica* (Burm) Merrill and *Tylophora dalzellii* Hook. f.

KOPPAKA V. RAO*, RICHARD A. WILSON, and BERNICE CUMMINGS

Abstract □ During a reexamination of the alkaloidal constituents of *Tylophora indica*, three new alkaloids (designated A, B, and C) were isolated. Analytical and spectral data indicate that these alkaloids are related to tylophorine and tylophorinine, which are also present in the plant. Alkaloid B is shown to be a desmethyltylophorine, and Alkaloid C is shown to be a desmethyltylophorinine. *T. dalzellii* has a relatively low alkaloid content, and desmethyltylophorinine is the major constituent. A brief description of the antileukemic activity of desmethyltylophorinine is presented.

Keyphrases □ *Tylophora indica*—isolation of three new alkaloids □ *Tylophora dalzellii*—desmethyltylophorinine determined as major constituent □ Desmethyltylophorinine—isolated and identified from *Tylophora indica* and *Tylophora dalzellii* □ Desmethyltylophorine—isolated and identified from *Tylophora indica*

In a recent publication, isolation of six new alkaloids from *Tylophora crebriflora*, together with the known members tylocrebrine, tylophorine, and septicine, was described (1). Structures for the new members were proposed based on the dibenzo[*f,h*]pyrrolo[1,2*b*]isoquinoline skeleton with four or five oxygen-bearing substituents (2). Interest in these alkaloids arose because of the antitumor activity shown by some members of this group. In this connection, it seemed worthwhile to reexamine the alkaloidal constituents of the related species *T. indica* (Burm) Merrill. A related species, *T. dalzellii* Hook. f., had not been examined, so a brief study of it also was undertaken.



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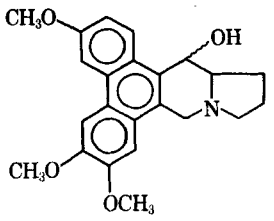

II

DISCUSSION

From *T. indica*, Govindachari *et al.* (3) isolated two alkaloids tylophorine and tylophorinine. They assigned Structures I and II for these compounds, respectively, and later recorded their syntheses (4–7).

The crude alkaloid from *T. indica*[1] was isolated by the following steps: extraction with 0.5% methanolic acetic acid, concentration,

[1] The plant material used was received from India. It was identified and a voucher specimen was preserved at Chas. Pfizer & Co., Inc., Maywood, N. J.

Table I—Properties of the Alkaloids of *T. indica*

	Alkaloid A		Alkaloid B		Alkaloid C	
Melting point	250–252° dec.		235–237° dec.		253–255° dec.	
Analysis	$C_{22}H_{23}NO_5 \cdot H_2O$		$C_{23}H_{25}NO_4$		$C_{22}H_{23}NO_4 \cdot {}^1/_2H_2O$	
	Calc.	Found	Calc.	Found	Calc.	Found
C	66.15	66.89	72.80	72.48	70.61	70.65
H	6.31	6.45	6.64	6.45	6.46	6.31
N	3.50	3.45	3.68	3.56	3.74	3.76
OCH_3	15.52 (2)	15.28	24.51 (3)	23.95	16.56 (2)	16.85
Molecular weight, *m/e*[a]	381		379		365	
UV spectrum	$\lambda_{max.}$	log *E*	$\lambda_{max.}$	log *E*	$\lambda_{max.}$	log *E*
	290	4.48	290	4.30	288	4.32
	257	4.70	257	4.80	258	4.65
Paper chromatographic behavior, R_f: formamide[b]–chloroform	0.2		0.5		0.1	

[a] Mass spectra were obtained on a Hitachi–Perkin-Elmer single-focusing instrument with 70 ev. as the ionizing voltage. [b] Whatman No. 1 filter paper moistened with a 30% solution of formamide in acetone, blotted, and developed with chloroform saturated with formamide, after the samples are spotted. Tylophorine and tylophorinine show R_f values of 0.7 and 0.3, respectively.

partition of the concentrate between 0.1 *N* HCl and ethyl acetate, extraction of the aqueous layer with chloroform at pH 9, concentration of the extract, and crystallization from methanol. It was freed from most of the tylophorine in the form of its slightly soluble hydrochloride. The mother liquors were then subjected to countercurrent distribution in the following system: 3% aqueous acetic acid–chloroform–*n*-butanol (3:2:1). The presence of five alkaloids was revealed, two of which were identified as tylophorine and tylophorinine. The remaining three appeared to be new and were designated as Alkaloids A, B, and C in the order of increasing polarity in the distribution. Together, these three alkaloids represented approximately 10–15% of the total alkaloid content; of the three, Alkaloid C was the major component. The analytical and physical data are given in Table I.

Alkaloid A has two phenolic groups and two methoxyls. The general similarity of its UV and IR spectra with those of tylophorine suggests that the substitution pattern of tylophorine may be present, with the fifth oxygen presumably being located at 14, in analogy with other members of this group.

Alkaloid B has three methoxyls. Its UV spectrum is very similar to that of tylophorine but shifts in basic solution, thus suggesting the presence of a phenolic hydroxyl. Methylation with diazomethane yields tylophorine. Alkaloid B is thus desmethyltylophorine, although the position of the phenolic group cannot be located unequivocally at this time.

Alkaloid C has two methoxyls, one phenolic hydroxyl, and one benzylic hydroxyl. It forms a diacetate, m.p. 225–227°.

Table II—NMR Spectra of Acetates of Tylophorinine and Alkaloid C

Proton	Tylophorinine	Alkaloid C	Remarks
1	τ 2.32, 2.17	2.35, 2.20	*AB* pattern
2	2.79, 2.94	2.79, 2.94	*AB* pattern, split (J = 2)
4	2.24	2.35	Split (J = 2)
5	2.24	1.77	Singlet
8	2.97	2.97	Singlet

Table III—Antileukemic Activity of Desmethyltylophorinine[a]

Dose, mg./kg.	Change in Body Weight	Increase in Survival Time, %
12	−2.2	135
8	−1.5	149
6	+0.2	145
4	+1.3	138
2.7	+2.3	123

[a] The author is indebted to Dr. T. J. McBride of the John L. Smith Memorial for Cancer Research, Chas. Pfizer & Co., Inc., Maywood, N. J., for the data in this table. The assay was carried out according to the protocols given in Cancer Chemotherapy Report No. 25, Dec. 1962. An increase in survival rate of 125% or higher is considered as positive activity.

Anal.—Calc. for $C_{26}H_{27}NO_6$: C, 69.47; H, 6.05; N, 3.11. Found: C, 69.05; H, 6.22; N, 3.25.

Its IR spectrum (bands at 1725 and 1760 cm.$^{-1}$) and NMR spectrum (3H peaks at τ 7.87 and 7.49) support the presence of an alcoholic and a phenolic acetyl group, respectively. Methylation with diazomethane leads to tylophorinine. Because of the low solubility in organic solvents, satisfactory NMR spectra could not be obtained. A comparison of the NMR spectra of the acetates of Alkaloid C and of tylophorinine is shown in Table II.

The spectra suggest that the phenolic hydroxyl is at either 3 or 6. A choice between the two is not possible from these data, and further work is planned.

Unlike the related species, *T. crebriflora* and *T. indica*, *T. dalzellii* is rather low in its alkaloid content. After routine extraction, the crude alkaloid was subjected to countercurrent distribution in the system already described. One major peak represented nearly all of the alkaloid. It was crystallized from chloroform–methanol, m.p. 253–255°.

Anal.—Calc. for $C_{22}H_{23}NO_4 \cdot H_2O$: C, 68.91; H, 6.57; N, 3.65; OCH_3 (2), 16.20. Found: C, 68.64; H, 6.37; N, 3.37; OCH_3, 15.60. Its melting point and analytical, spectral, and chromatographic data showed that it was identical with Alkaloid C of *T. indica*, desmethyltylophorinine.

Of the five alkaloids of *T. indica*, only Alkaloid C showed significant activity in murine leukemia (L-1210 system) (Table III).

REFERENCES

(1) K. V. Rao, *J. Pharm. Sci.*, **59**, 1501(1970).

(2) *Ibid.*, **59**, 1608(1970).

(3) T. R. Govindachari, B. R. Pai, and K. Nagarajan, *J. Chem. Soc.*, **1954**, 2669.

(4) T. R. Govindachari, M. V. Lakshmikantham, K. Nagarajan, and B. R. Pai, *Chem. Ind.*, **1957**, 1484.

(5) T. R. Govindachari, B. R. Pai, S. Rajappa, and N. Viswanathan, *Ibid.*, **1959**, 950.

(6) T. R. Govindachari, M. V. Lakshmikantham, B. R. Pai, and S. Rajappa, *Tetrahedron*, **9**, 53(1960).

(7) T. R. Govindachari, B. R. Pai, S. Prabhakar, and T. S. Savitri, *ibid.*, **21**, 2573(1965).

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