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REFERENCES

1. Abeywickrama, B. A. *Flora of Ceylon* (Revised hand book), Vol. I, p. 12.
2. Gilbert, B., Brissolese, J. A., Finch, N., Taylor, W. I., Budzikiewicz, H., Wilson, J. M. and Djerassi, C. (1963) *J. Am. Chem. Soc.* **85**, 1523.
3. Shamma, M. and Foley, K. F. (1967) *J. Am. Chem. Soc.* **32**, 4141.
4. Bax, A. and Freeman, R. (1981) *J. Magn. Reson.* **42**, 64.
5. Aue, W. P., Karhan, J. and Ernst, R. R. (1976) *J. Chem. Phys.* **64**, 4226.
6. Hofle, G., Heinstein, P., Stockigt, J. and Zenk, M. H. (1980) *Planta Med.* **40**, 120.
7. Phillipson, J. D. and Hemingway, S. R. (1973) *Phytochemistry* **12**, 1481.
8. Beecham, A. F., Hart, N. K., Johns, S. R. and Lamberton, J. A. (1968) *Aust. J. Chem.* **21**, 491.
9. Noggle, J. H. and Schirmer, R. F. (1971) *The Nuclear Overhauser Effect*. Academic Press, New York.
10. Sanders, J. K. M. and Mersh, J. D. (1982) *Prog. NMR Spectrosc.* **13**, 353.
11. Doddrell, D. M., Pegg, D. T. and Bendall, M. R. (1982) *J. Magn. Reson.* **48**, 323.
12. Wenkert, E., Bindra, J. S., Chang, C. J., Cohran, D. W. and Schell, F. M. (1974) *Acc. Chem. Res.* **7**, 46.
13. Wenkert, E., Chang, C. J., Chawla, H. P. S., Cochran, D. W., Hagaman, E. W., King, J. C. and Orito, K. (1976) *J. Am. Chem. Soc.* **98**, 3045.

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ALKALOIDS OF *ALSTONIA MACROPHYLLA*

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Key Word Index—*Alstonia macrophylla*: Apocynaceae; stem bark; leaves; indole alkaloids; ^{13}C NMR; DEPT.

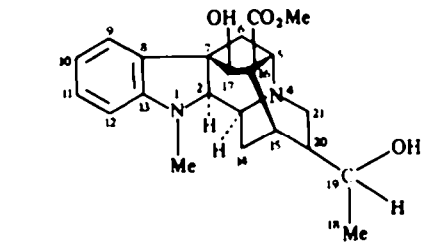
Abstract— A new alkaloid, 19-hydroxyvincamajine, has been isolated from the leaves of *Alstonia macrophylla*. In addition to this, eight other indole alkaloids, alstonerine, alstophylline, macralstonine, anhydromacralstonine, talcarpine, vincamajine, vincorine and cabucraline, were also isolated and identified from the bark and leaves of *A. macrophylla* of Sri Lanka. The last five alkaloids have been isolated for the first time from this species and the ^{13} NMR of alstonerine and alstophylline are reported.

INTRODUCTION

Alstonia macrophylla is a common plant in Sri Lanka. Several studies on this species growing in other countries have been reported [1–10] and the plant is used in medicinal preparations in the Philippines [2]. However, little previous work has been done on *A. macrophylla* growing in Sri Lanka. Therefore, an investigation of its alkaloidal constituents was initiated.

RESULTS AND DISCUSSION

Four alkaloids, namely talcarpine, alstonerine, macralstonine and anhydromacralstonine, have been isolated and identified from the bark of *A. macrophylla* [12], and vincorine, vincamajine, cabucraline, alstophylline from the leaves, by comparing their spectra with those of known compounds. A new vincamajine derivative was also isolated from the leaves of the plant. Its IR spectrum contained an acetyl band at 1730 cm^{-1}, a band at 740 cm^{-1} characteristic of an *ortho* disubstituted benzene and another band at 1608 cm^{-1}. The ^{1}H NMR spectrum confirmed the presence of an acetyl group at δ3.7 (3H, *s*), a *N*-methyl group at δ2.6 and showed a doublet at δ1.0 (J = 7 Hz). The NMR spectrum of the new compound therefore shows a close resemblence to that of vincamajine [13 15] except for the doublet at δ1.0. The doublet at δ1.0 (J = 7 Hz) can be assigned to a C-19 methyl group. The mass spectrum showed the $[M]^+$ at m/z 384 and a peak at m/z 360 which can be formed by the loss of water from the $[M]^+$. Peaks in the spectrum of lower m/z values can be explained by comparison with the fragmentation patterns established for vincamajine. The fragment ion at m/z 222 is characteristic of alkaloids belonging to the quebrachidine group and the ion at m/z 190 is formed by the loss of MeOH from the former ion. These spectral data indicate that the compound is 19-hydroxyvincamajine (**1**) which is a new alkaloid. Recently *O*-



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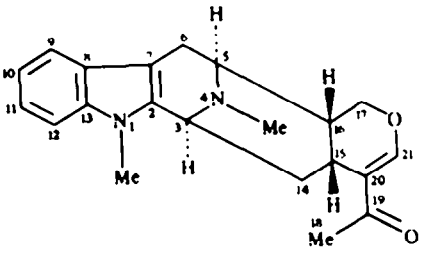

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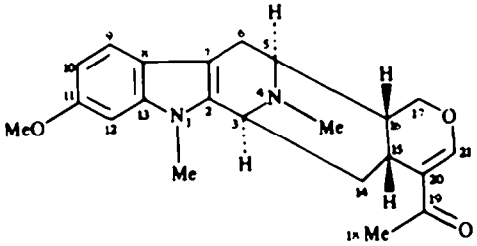

3

benzoylvincamajine [16] has been obtained in very low yields from the leaves of *A. macrophylla*.

Anhydromacralstonine, which is easily formed from macralstonine by treatment with hydrochloric acid, could be an artifact. Therefore the crude ethanol extract was checked and that too showed the presence of this compound, indicating that it is not formed during the acid treatment stage.

The ^{13}C NMR spectra (Table 1) of alstonerine (**2**) and alstophylline (**3**) were in agreement with the structures proposed earlier for these alkaloids. The assignments (Table 1) were made by comparison with other related indole alkaloids [18] and these assignments were confirmed by employing DEPT. Pulse sequences with the last polarization pulse O being adjusted to 135°, 90° and 45° [19] to distinguish between methyl, methylene, methine and quarternary carbons. The ^{13}C NMR spectra of both compounds were found to be similar, the main difference being in the aromatic region where C-10 and C-12 of alstophylline were found to be downfield by 5.51 and 10.1 ppm, respectively, in comparison with alstonerine on account of the presence of the OMe group at C-11 [20].

The isolation of vincamajine, its *O*-benzoyl and 3,4,5-trimethoxycinnamoyl derivatives from *A. constricta* [14] is of interest because they indicate the chemotaxonomic relationships between the various species of *Alstonia*. The *bis* indole alkaloid macralstonine has been previously reported [5] from *A. macrophylla*. Alstonerine, however, has been reported [1] as demethoxy alstophylline while talcarpine which is a constituent of *Pleiocarpa talboti* [21] has not been reported from *Alstonia*. The presence of macroline alkaloids in genera other than *Alstonia* is important because these characteristic compounds indicate the relationship between the genera and they can also be used as markers in chemotaxonomic studies. The

Table 1. ^{13}C NMR assignments of alstonerine and alstophylline (75 MHz, $CDCl_3$)

Carbon No.	Alstonerine	Multiplicity DEPT	Alstophylline	Multiplicity DEPT
2	137.39	-C	138.87	-C
3	54.86	CH	55.81	CH
5	54.02	CH	55.81	CH
6	22.96	CH_2	23.97	CH_2
7	105.93	-C-	104.77	-C
8	126.50	C	126.50	C-
9	117.91	CH	118.92	-CH
10	121.02	CH	110.92	CH
11	118.87	CH	157.93	-CH
12	109.76	CH	93.56	CH
13	137.39	-C	138.87	C
14	38.67	CH	38.67	CH
15	32.29	CH_2	29.72	CH_2
16	41.75	CH	40.60	CH
17	67.75	CH_2	66.01	CH_2
18	22.42	CH_3	22.42	CH_2
19	195.44	C=O	195.35	C=O
20	W	-C-	W	-C
21	157.45	-CH	157.93	-CH
N-CH_3	29.12	CH_3	29.47	CH_3
N-CH_3	25.04	CH_3	25.01	CH_3

W Weak, the signal could not be established with certainty.

common structural element in all the leaf alkaloids reported here is macroline. The major alkaloids of the bark of *A. muelleriana* [22] are also derived from macroline. This confirms the close relationship between *A. muelleriana and A. macrophylla.*

Vincorine, vincamajine, cabucraline and alstophylline are reported for the first time from *A. macrophylla* leaf, although the first three compounds have been isolated from other *Alstonia* species [23] and alstophylline from the bark of *A. macrophylla* [17].

EXPERIMENTAL

Leaves and bark of *A. macrophylla* Wall. were collected from trees growing in Colombo district and were identified by Prof. S. Balasubramanium, Dept. of Botany, University of Peradeniya.

Extraction and separation of alkaloids. Dried powdered plant material was extd with 70% EtOH by percolation at room temp. The EtOH extract was concd, acidified with dil. HCl, filtered and defatted by extracting with petrol. It was then basified and extd repeatedly with $CHCl_3$ to obtain tertiary bases, bark (3%) and leaves (0.8%). The extracts were fractionated by CC using a $CHCl_3$ MeOH mixture. The fractions were further sepd by TLC to isolate individual alkaloids.

Identification of alkaloids. Isolated compounds were identified by comparing their physical and spectral data with those of known compounds. Cabucraline was also identified by comparison with an authentic sample. ^{13}C NMR spectra were recorded at the H. E. J. Research Institute of Chemistry, University of Karachi.

19-*Hydroxyvincamajine* (1). UV λ_{max} nm (log ε): 247 (3.8), 175 (3.5). ^1H NMR ($CDCl_3$, 60 MHz) δ 7.1–8, 5.3 (1H, *s*, H-17), 3.7 (3H, *s*, MeCO), 3.6 (1H, *d*, H-15), 3.5 (2H, *m*, H-21), 3.3 (1H, *m*, H-3), 3.2 (1H, *d*, H-2), 2.6 (3H, *s*, *N*-Me), 1.0 (3H, *d*, Me–C–O); IR ν_{max} cm^{-1} 3040, 2980, 1730, 1610; MS (70 eV) *m/z* (rel. int.%): 384 $[M]^+$ (1), 366 (17), 349 (1), 222 (29), 190 (54), 157 (100), 144 (81).

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REFERENCES

1. Manalo, G. D. (1967) *Nat. Appl. Sci. Bull.* **20**, 225.
2. Manalo, G. D. (1968) *Philippine J. Sci.* **97**, 259.
3. Banerji, A. and Chakrabarty, M. (1973) *Indian J. Chem.* **11**, 706.
4. Banerji, A., Chakrabarty, M. and Mukherjee, B. (1972) *Phytochemistry* **11**, 2605.
5. Kishi, T., Hesse, M., Vetter, W., Gemenden, C. W., Taylor, W. I. and Schmid, H. (1966) *Helv. Chim. Acta* **49**, 946.
6. Sharp, T. M. (1934) *J. Chem. Soc.* 1227.
7. Waldner, E. E., Hesse, M., Taylor, W. I. and Schmid, H. (1967) *Helv. Chim. Acta* **50**, 1926.
8. Khan, Z. M., Hesse, M. and Schmid, H. (1967) *Helv. Chim. Acta* **50**, 1002.
9. Wolfes, H. and Niggli, A. (1967) *Helv. Chim. Acta* **50**, 1011.
10. Hesse, M., Hurzeler, H., Gemenden, C. W., Joshi, B. S., Taylor, W. I. and Schmid, H. (1965) *Helv. Chim. Acta* **48**, 689.
11. Mayerl, F. and Hesse, M. (1978) *Helv. Chim. Acta* **61**, 337.
12. Hettiarachchi, C. K., Arambewella, L. S. R. and De Silva, K. T. D. (1984) *Fifth Asian Symposium on Medicinal Plants and Spices* , Abstr. C17, 119.
13. Janot, M. M. and Le Men, J. (1955) *Compt. rend* **241**, 767.
14. Crow, W. D., Hancox, W., Johns, S. R. and Lamberton, J. A. (1970) *Aust. J. Chem.* **23**, 2489.
15. Aynilian, G. H., Bell, C. L., Farnswarth, N. R. and Abraham, D. J. (1974) *Lloydia* **37**, 589.
16. Mukherjee, B., Ray, A. B., Chatterjee, A. and Das, B. C. (1969) *Chem. Ind.* 1387.
17. Kishi, T., Hesse, M., Gemenden, C. W., Taylor, W. I. and Schmid, H. (1965) *Helv. Chim. Acta* **48**, 1349.
18. Das, B. C., Cosson, J. P., Lukais, G. and Potier, P. (1974) *Tetrahedron Letters* 4299.
19. Doddrell, D. M., Pegg, D. and Bendall, M. R. (1982) *J. Magn. Res.* **48**, 323.
20. Kalamaras, S. M., Sevenet, J., Thal, C. and Potier, P. (1975) *Phytochemistry* **14**, 1637.
21. Naranjo, J., Pinar, M., Hesse, M. and Schmid, H. (1972) *Helv. Chim. Acta* **55**, 752.
22. Burke, D. E., Cook, G. A., Haller, K. G., Lazar, H. A. and Le Quesne, W. (1973) *Phytochemistry* **12**, 1467.
23. Guillanne, D., Morfaux, A. M., Richard, B., Massiot, G., Le Men Oliver, Pusset, L. and Sevenet, T. (1984) *Phytochemistry* **23**, 2407.