Plant Antitumour Agents: Colubrinol Acetate and Colubrinol, Antileukaemic Ansa Macrolides from *Colubrina texensis*[1]

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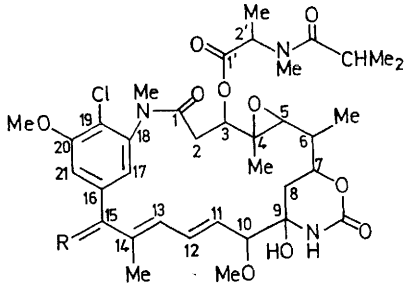
Summary The isolation and structures of two new antileukaemic ansa macrolides, colubrinol acetate (**1**) and colubrinol (**2**), have been described.

RECENTLY the isolation and structure of antileukaemic ansa macrolides from *Maytenus ovatus*[2] and *Maytenus buchananii*[3] (Celastraceae) have been reported. We have now isolated two new tumour inhibitory† ansa macrolides, colubrinol acetate (**1**) and colubrinol (**2**), from *Colubrina texensis* Gray (Rhamnaceae), along with the known ansa macrolide maytanbutine (**3**).[3]



(1) R = H, OAc (3) R = H_2
(2) R = H, OH (4) R = O

Fractionation of the viscous residue from an alcohol extract, guided by assay in 9KB and P388 leukaemia system† showed that the antitumour activity was concentrated successively in the chloroform layer of a chloroform–water partition and the methanol layer of a 10% aqueous methanol–petroleum partition. Three successive applications of column chromatography to the residue from the aqueous methanol layer on Florisil, Sephadex LH-20, and neutral alumina (activity IV), followed by repeated preparative t.l.c. on alumina and silica gel, gave colubrinol acetate (**1**)‡ (0·00005%), M^+ 777·3249, m.p. 179—182°, $[\alpha]_D^{22}$ −127° (*c* 0·073 in $CHCl_3$); colubrinol (**2**) (0·00003% *m/e* 674·2929 [*M* − 61 (H_2O + $HNCO^2$)], m.p. 194—196° $[\alpha]_D^{22}$ −94° (*c* 0·035 in $CHCl_3$); and matanbutine[3] (**3**) (0·00002%), M^+ 719·3191 m.p. 178—180° (lit.,[3] 170—171°), $[\alpha]_D^{22}$ −126° (*c* 0·049 in $CHCl_3$). The identity of (**3**) was established by comparison of its $[\alpha]_D$, i.r., u.v., n.m.r., and mass spectral properties with those previously reported.[3] The i.r. and u.v. spectra of (**1**) and (**2**) were very similar to those of (**3**).

The molecular formulae of compounds (**1**)—(**3**) suggested that (**1**) and (**2**) were closely related to (**3**) but contained an additional acetoxy or hydroxy-group, respectively. In agreement with this the ester carbonyl absorption at 1748 cm^{-1} for (**1**) was more intense than that for (**2**) or (**3**). Furthermore, mild base-catalysed methanolysis[4] of (**1**) gave (**2**), and acetylation of the latter gave (**1**), indicating that the hydroxy-function in (**2**) was at the same position as the acetoxy-group in (**1**).

The n.m.r. and mass spectra of these three compounds revealed the presence of the same ester side chain in all. The additional acetoxy or hydroxy-function must, therefore, be located in the macrocycle.

The assignment of the acetoxy-function to C-14 is based on n.m.r. and chemical evidence. In the n.m.r. spectrum of maytanbutine (**3**) the doublets at δ 3·13 and 3·67 (*J* 13 Hz) have been assigned to the protons at C-15.[3] Both these signals are absent in the n.m.r. spectra of (**1**) and (**2**). Furthermore, the singlet at δ 5·48, which could be assigned to the C-15 proton in (**2**), is shifted downfield to δ 7·01 in (**1**). Manganese dioxide[5] oxidation of (**2**) gave the ketone (**4**).§

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† Both (**1**) and (**2**) exhibited confirmed activity against P388 lymphocytic leukaemia at the microgram per kilogram level and cytotoxicity (ED_{50}) against KB cell culture at 10^{-4}—10^{-5} μg ml^{-1}. Cytotoxicity and *in vivo* activity tests were carried out under the auspices of the National Cancer Institute by the procedures described in *Cancer Chemother. Reports*, 1962, **25**, 1.

‡ Although homogeneous by t.l.c. in a number of different solvent systems, all attempts to crystallize this compound were unsuccessful.

§ Because of the extremely limited quantity of (**2**), the identity of the oxidation product (**4**) was established by high resolution mass spectroscopy only: *m/e* 672·2839 [*M* − 61 (H_2O + $HNCO^2$)].

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