Phytochemistry, Vol. 27, No. 7, pp. 2350 2352, 1988.
Printed in Great Britain.

0031 9422/88 $3.00 + 0.00

ISOLATION AND STRUCTURE OF AN A-RING CONTRACTED TRITERPENOID FROM *COLUBRINA TEXENSIS* TISSUE CULTURE

R. L. BAXTER* and M. D. WALKINSHAW†

Chemistry Department, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, U.K.

(*Received* 12 *October* 1987)

Key Word Index—*Colubrina texensis*; Rhamnaceae, suspension cultures; pentacyclic triterpene acid; colubrinic acid; X-ray crystallography.

Abstract—An A-ring contracted triterpenoid produced by suspension cultures of *Colubrina texensis* was shown to be 2β-formyl-3α-hydroxy-nor-A(1)-lup-20(29)-en-28-oic acid by X-ray crystallography. The methyl ester derivative was shown to be identical with methyl colubrinate, a compound previously isolated from a methylated acidic fraction from *C. granulosa* heartwood.

INTRODUCTION

Over the past decade there has been great interest in the potential use of plant tissue cultures of medicinally important plants for the production of natural products having pharmacological activity [1–4]. While this potential has been realized in a few instances [5], many plant cell cultures fail to exhibit the diversity and stability of secondary metabolite production found in the intact plant. More frequently the desired product is produced in much smaller quantities than in the plant or alternatively biosynthetic precursors are accumulated at the expense of the final product [6].

The maytansenoids, a family of closely related antileukaemic ansa macrolides, have been isolated principally from the family Celastraceae [7–10]. Three compounds of this series, maytanbutine, colubrinol and colibrinol acetate have been reported as constituents of the plant *Colubrina texensis* (Rhamnaceae) [11]. We have recently examined tissue cultures of *C. texensis* as a potential source of these materials and report here the isolation and structure of colubrinic acid (**1a**) an A-ring modified lupane-type triterpenoid, produced by suspension cultures of this plant.‡

RESULTS AND DISCUSSION

An ethanol extract of freeze-dried cells from a 21 day culture was fractionated by successive column and thin layer chromatography to afford colubrinic acid (**1a**, 0.05%), $C_{30}H_{46}O_4$, mp 282–284°. Its IR spectrum (KBr) indicated the presence of two carbonyl groups (1718 and 1700 cm^{-1}) and of hydroxyl groups (3385 cm^{-1}). Assignment of the carbonyl frequencies to carboxylic acid and aldehyde functions was indicated by ^{13}C NMR resonances at δ 177.6 (*s*) and 205.5 (*d*). The ^{13}C NMR spectrum showed the presence of an isopropylidene group [methyl singlet at δ 25.2 and sp^2 carbons at 108.9 (*t*) and 150.0 (*s*)] and a secondary alcohol [79.7 (*d*)]. The 500 MHz ^1H NMR spectrum showed five angular methyl groups and an allylic methyl group. Analysis of coupling constant data, supported by decoupling experiments, revealed two isolated ^1H–^1H coupling sequences corresponding to H-1→ H-3 and H-9→H-22 portions of the molecule shown in Fig. 1. The resonance of the H-13 and H-19 methine protons at high frequency (δ 2.30 and 3.02, respectively) can be ascribed to the shielding effect of the axial C-17 carboxylic acid group whille the appearance of the 11α-equatorial proton resonance at δ 2.24 suggests that this is shielded by the aldehyde carbonyl group and thus indicates that the formyl group attached to the C-2 position has the β-orientation. In $^2H_6$-DMSO solution the hydroxyl proton resonates as a doublet (δ 4.73, ^3J = 6.6 Hz) coupled to H-3 and the aldehydic proton is shifted to 7.93. The structure and configuration of **1a** was determined by single crystal X-ray diffraction.

In the crystal structure, shown in Fig. 2, neither C–C bond lengths nor bond angles are exceptional. The bond lengths between C-20/C-29 (1.37 Å) and C-20/C-30 (1.46 Å) suggest that C-29 is the methylene carbon of the isopropylene group although the high thermal parameters for both C-29 and C-30 indicate that this substituent is disordered about the C-19/C-20 bond. The ring system is slightly flattened around the C-9/C-10 and C-8/C-9 bonds presumably as a result of steric repulsion between the C-10 and C-8 axial methyl groups.

The similarity of spectroscopic data obtained for **1a** to that reported for methyl colubrinate, a compound isolated following esterification of a crude acidic fraction

*Author to whom correspondence should be addressed.

†Present address: Pharmaceutical Division, Sandoz AG, CH-4002 Basel, Switzerland.

‡No significant level of cytotoxicity against KB (human carcinoma of the nasopharanx) nor HeLa cells [12] was detected for either cell extracts of *C. texensis* tissue cultures or for colubrinic acid.

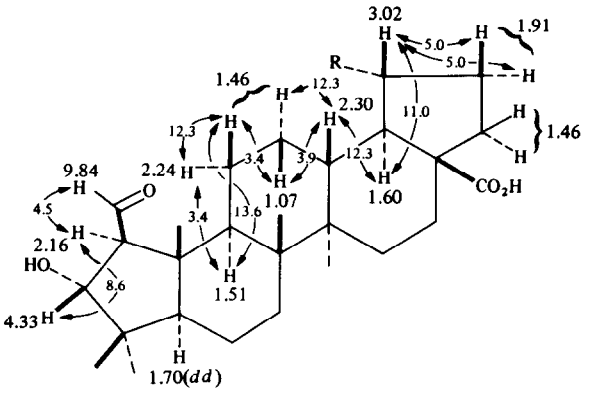


Fig. 1. Structure of **1a** showing the ^1H–^1H connectivities derived from ^1H NMR data. Chemical shifts are in ppm relative to tetramethylsilane and coupling constants are given in Hz.

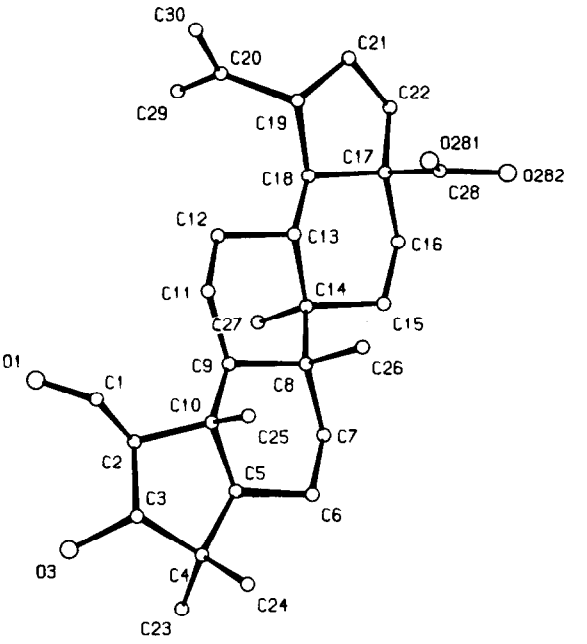
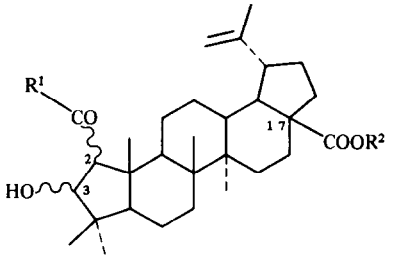


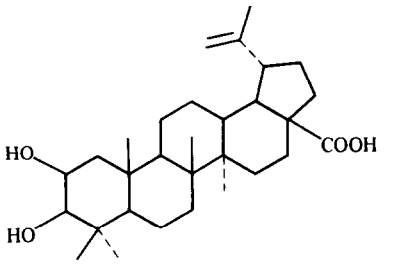
Fig. 2. Molecular structure of **1a** as found in the crystal.

derived from *C. granulosa* heartwood [13], suggested that **1a** and the free carboxylic acid from *C. granulosa* might be identical. On the basis of ^1H NMR data the 2β,3α-stereochemistry has been suggested for methyl colubrinate [13]. Esterification of **1a** was carried out by treatment with ethereal diazomethane to give the corresponding methyl ester (**1b**) which proved identical in all respects with a sample of methyl colubrinate confirming the structure previously suggested for this compound [13].

Colubrinic acid belongs to the rare group of A-ring contracted lupane triterpenoids and is closely related to the dicarboxylic acid ceanothic acid (**1c**) isolated from a number of plants f the family Rhamnaceae [13–16]. The formation of **1a** can be conjectured to involve a straightforward aldol condensation of a 2,3-*seco*-dialdehyde derived by oxidative cleavage of alphitolic acid (**2**).



1a (2β,3α) R^1 = H, R^2 = H
1b (2β,3α) R^1 = H, R^2 = Me
1c (2α,3β) R^1 = OH, R^2 = H



2

EXPERIMENTAL

Callus cultures of *C. texensis*, initiated from leaf explants, were maintained on SH agar [17]. Suspension cultures were initiated by transfer to SH medium and grown under constant illumination at 17° on an orbital shaker at 120 rev/min. For preparative purposes cultures were grown in a stirred, aerated 5 l fermenter for 21 days. Cells were harvested by filtration, freeze-dried (40 g) and extracted with EtOH (500 ml) at room temp. The concd extract was diluted with H_2O (1:1, 200 ml) and extracted with $CHCl_3$ (200 ml × 2). Column chromatography of the concd $CHCl_3$ extract on silica gel employing increasing proportions of Et_2O in toluene as an eluant afforded a triterpenoid rich fraction (80 mg) which was subjected to prep. TLC on silica gel using MeOH–$CHCl_3$ (1:9) to afford colubrinic acid (**1a**, 22 mg). Colubrinic acid crystallized from MeOH as colourless prisms, mp 282–284°. EIMS *m/z* 470.3394 $[M]^+$ ($C_{30}H_{46}O_4$ requires 470.3396), 452 $[M-H_2O]^+$, 355, 248, 189, 174, 69: IR ν_{max} cm^{-1}: 3385 (br), 1718, 1700; ^1H NMR (500 MHz, $^2H_6$-acetone): δ 0.90(9), 0.91(3), 0.96, 1.02, 1.04 (15H, 5*s*, 5 × Me-C-), 1.07 (1H, *m*, 12β-H), 1.46 (4H, *m*, 11β-H, 12β-H, 6α-H, 6β-H), 1.49 (2H, *m*, 22-H_2), 1.54 (1H, *dd*, *J* = 13.6, 3.5 Hz, H-9), 1.60 (1H, *dd*, *J* = 12.3, 11.0 Hz, 18-H), 1.68 (3H, *s*, Me-C=), 1.70 (1H, *dd*, *J* = 13.0, 3.4 Hz, 5-H), 1.91 (2H, *m*, 21-H_2), 2.16 (1H, *dd*, *J* = 8.9, 4.5 Hz, 2-H), 2.24 (1H, *dt*, *J* = 12.3, 3.4 Hz, 11α-H), 2.30 (1H, *ddd*, *J* = 12.3, 12.3, 3.9 Hz, 13-H), 3.02 (1H, *ddd*, *J* = 11.0, 5.0, 5.0 Hz, 19-H), 4.33 (1H, *d*, *J* = 8.8 Hz, 3-H), 4.58 (1H, *d*, *J* = 1.5 Hz, 29-H), 4.70 (1H, *d*, *J* = 1.5 Hz, 29-H), 9.84 (1H, *d*, *J* = 4.5 Hz, *CHO*); ^1H NMR (500 MHz, $^2H_6$-DMSO) δ 0.82 (6H, *s*, 2 × Me-C-), 0.89, 0.94, 0.95 (9H, 3*s*, 3 × Me-C-), 0.9-1.1 (6H, *m*), 1.2–1.5 (10H, *m*), 1.65 (3H, *s*, Me-C=), 1.78 (2H, *m*, 21-H_2), 2.04 (1H, *dd*, *J* = 8.6, 4.7 Hz, 2-H), 2.11 (1H, *m*, 11α-H), 2.17 (1H, *ddd*, *J* = 12.0, 12.0, 4.0 Hz, 13-H), 2.94 (1H, *m*, 19-H), 4.18 (1H, *brt*, *J* = 8,4 Hz, 3-H), 4.56 (1H, *s*, 30-H), 4.69 (1H, *s*, 30-H), 4.73 (1H, *d*, *J* = 6.6 Hz, *OH*), 7.93 (1H, *d*, *J* = 4.6 Hz, *CHO*); ^{13}C NMR (75 MHz, $^2H_5$-pyridine)

δ 13.7, 13.9, 15.9, 17.9, 18.3, 25.2 (6 × Me), 17.4, 23.7, 24.5, 29.2, 30.1, 31.8, 33.5, 36.5, 108.9 (9 × CH_2), 37.3, 47.1, 48.6, 49.3, 61.8, 72.7, 79.7, 205.5 (8 × CH), 40.1, 41.1, 41.9, 46.7, 55.4, 150.0 and 177.6 (7 × C).

Methyl colubrinate (**1b**): Colubrinic acid (5 mg) in THF (0.5 ml) at 0° was treated wtih an excess of CH_2N_2 in Et_2O (0.5 ml) for 5 min. The unreacted CH_2N_2 was destroyed with HOAC and the soln evapd to dryness *in vacuo*. Crystallization of the residue from Et_2O–hexanes afforded methyl colubrinate (**1b**, 3 mg), mp 178–181°, which proved identical (mmp, IR, TLC) with an authentic sample.

Crystal structure of compound **1a**: Colubrinic acid crystallized in the space group $C2$, $a = 17.005$ (5), $= 18.304$ (7), $c = 10.051$ (4) Å, $\beta = 119.26$ (3)°, $U = 2729.3 A^3$, $Z = 4$, $D_c = 1.145$ gcm^3. A crystal of dimensions 0.25 × 0.25 × 0.07 mm was mounted on a glass fibre and data collected out to $\theta = 20°$) ($h_{max} = -16$ to 16, $k_{max} = 17$, $l_{max} = 9$) on a Nonius CAD4 diffractometer using M_oK_α graphite monochromated radiation. Of the 1323 reflections measured only 579 with $I > 2\sigma(I)$ and were classed as observed. No absorption correction was applied.

The structure was solved using direct methods [18] and refined using SHELX76 [19]. All hydrogen atoms (except five on the partially disordered isopropyl group and those on O-3 and O-282) were included in idealised positions with fixed C-H bond lengths of 1.08 Å and fixed temperature factors of 0.08 Å^2. Isotropic refinement of all non-hydrogen atoms gave a final $R = 0.077$. Unit weights were found to give the best analysis of variance in range of and $|F|$. The average shift over error in the final cycle of full matrix least squares refinement was less than 0.1 and the largest minimum and maximum on the final differences Fourier map were 0.3 eÅ^{-3} and -0.2 Å^{-3} respectively. With a weak data set of 579 observed reflections and a total of 151 thermal and positional parameters it was not possible to carry out any anisotropic refinement.

Acknowledgment—We thank Dr J. N. Roitman for a sample of methyl colubrinate.

REFERENCES

1. Misawa, M. (1977) in *Plant Tissue Culture and its Biotechnological Applications* (Barz, W., Reinhard, E. and Zenk, M. H., eds), pp. 17–26. Springer, Berlin.
2. Zenk, M.H. (1978) in *Frontiers of Plant Tissue Culture*, 4*th IAPTC Cong. Proc.* (Thorpe, T.A., ed.), pp. 1–13. University of Calgary, Calgary.
3. Kurz, W. G. W. and Constabel, F. (1979) in *Microbial Technology* (Peppler, H. J. and Perlman, D., eds), 2nd Edn, Vol. 1, pp. 389–416. Academic Press, New York.
4. Stava, E. J. (1980) *Plant Tissue Culture as a Source of Biochemicals*. C.R.C. Press, Florida.
5. Yamada, Y. and Fujita, Y. (1983) in *Handbook of Plant Cell Culture* (Evans, D. A., Sharp, W. R., Ammirato, P. V. and Yamada, Y., eds), Vol. 1, pp. 717–728. MacMillan, New York.
6. Yeoman, M. M., Miedzybrodzka, M. B., Lindsey, K. and McLauchlan, W. R. (1980) in *Plant Cell Cultures: Results and Perspectives* (Sala, F., Parisi, G., Ceila, R. and Ciferri, O., eds) pp. 327–343. Elsevier/North Holland, Amsterdam.
7. Kupchan, S. M., Komoda, Y., Court, W. A., Thomas, G. J., Smith, R. M., Karim, A., Gilmore, C. J., Haltiwanger, R. C. and Bryan, R. F. (1972) *J. Am. Chem. Soc.* **94**, 1354.
8. Kupchan, S. M., Komoda, Y., Thomas, G. J. and Hintz, H. P. J. (1972) *J. Chem. Soc., Chem. Commun.* 1065.
9. Kupchan, S. M., Branfman, A. R., Sneden, A. T., Verma, A. K., Dailey, R. G., Komoda, Y. and Nagao, Y. (1975) *J. Am. Chem. Soc.*, **97**, 5294.
10. Kupchan, S. M. (1976) *Cancer Chemother. Rep.* **60**, 1115.
11. Wani, M. C., Taylor, H. L. and Wall, M. E. (1973) *J. Chem. Soc., Chem. Commun.* 390.
12. Geran, R. I., Greenberg, N. J., McDonald, M. M. Schumacher, A. M. and Abbott, B. J. (1972) *Cancer Chemother. Rep.* **3**, 1.
13. Roitman, J. N. and Jurd, L. (1978) *Phytochemistry* **17**, 491.
14. Julian, P. L., Pikl, J. and Dawson, R. (1938) *J. Am. chem. Soc.*, **60**, 77.
15. Boyer, J. P., Eade, R. A., Locksley, H. and Simes, J. J. H. (1958) *Aust. J. Chem.* **11**, 236.
16. Guise, G. B., Ritchie, E. and Taylor, W. C. (1962) *Aust. J. Chem.* **15**, 314.
17. Schenk, R. V. and Hilderbrandt, A. C. (1972) *Can. J. Botany* **50**, 199.
18. Sheldrick, G. M. (1984) SHELX84, Program for Crystal Structure Solution, Univ. Göttingen, F.R.G..
19. Sheldrick, G. M. (1976) SHELX76, Program for Crystal Structure Refinement, Univ. Cambridge, U.K.