CONSTITUENTS OF *NEALCHORNEA YAPURENIS* (EUPHORBIACEAE)

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ABSTRACT.—Extracts of *Nealchornea yapurensis* Huber (Euphorbiaceae) displayed cytotoxic activity; from the active fractions, five known compounds acetylaleuritolic acid, scopoletin, hexacosanoic acid, β-sitosterol and β-sitosterol glucoside were obtained. None of these compounds displayed cytotoxic activity.

The family Euphorbiaceae has recently been the source of several interesting compounds or classes of compounds showing anticancer activity including jatrophone (1) and the phorbol esters (2–5). We were, therefore, interested in examining the plant *Nealchornea yapurensis* Huber (Euphorbiaceae) which had shown activity in the P-388 lymphocytic leukemia system *in vivo*.[1]

A recollection of the root material of *N. yapurensis* from Peru failed to show antitumor activity but did show activity in the Eagles carcinoma of the nasopharynx test system in cell culture.

Extensive fractionation of the chloroform soluble fraction (ED_{50} 4.5 μg/ml) with concomitant bioassay gave several active fractions. But these were either too small or too complex to be further studied with a reasonable assurance of obtaining sufficient compound for evaluation and identification. In the course of these studies, five compounds **1**, **2**, **3**, **4** and **5** were isolated and shown to be inactive.

EXPERIMENTAL[2]

PLANT MATERIAL.—The roots of *Nealchornea yapurensis* used in this study were collected in Peru in October 1975. A voucher specimen representing the collection is deposited in the Herbarium of the National Arboretum, U.S. Department of Agriculture, Washington, D.C.

EXTRACTION AND FRACTIONATION.—The dried and milled root material (29.5 kg) was thoroughly extracted successively with light petroleum and methanol. Evaporation of the methanol extract *in vacuo* afforded a residue (940.5 g) which was partitioned between water (4 liters) and chloroform (3 liters) to afford, after processing, 99.7 g of chloroform-soluble material.

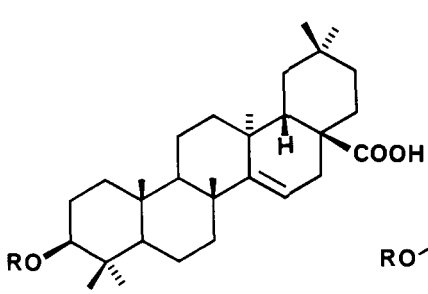
CHROMATOGRAPHIC SEPARATION.—The chloroform-soluble fraction (61.9 g) was chromatographed on a column of silica gel PF 254[3] (750 g). Elution with benzene, benzene-chloroform (3:1), benzene-chloroform (1:1) and chloroform gave fractions 1–31, 32–36, 37–42 and 43–61 respectively.

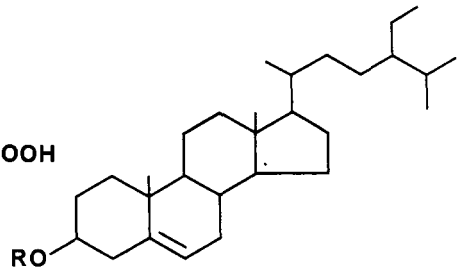
ISOLATION AND IDENTIFICATION OF ACETYLALEURITOLIC ACID (**1**).—Fraction 3 (2.71 g) on crystallization from n-hexane yielded acetylaleuritolic acid (**1**) as white needles (210 mg,

[1]The extracts, fractions, and compounds were tested under the auspices of the Drug Research and Development Program of the National Cancer Institute (6). An isolate is considered active if it shows an ED_{50} of 4 μg/ml or less in the 9KB or P-388 cell culture system *in vitro* or a T/C of greater than 130% in the P-388 lymphocytic leukemia system *in vivo*.

[2]Melting points were determined with a Kofler hot plate and are uncorrected. The uv spectra were obtained with a Beckman model DB-G grating spectrophotometer. The ir spectra were determined with a Beckman, model 18-A spectrophotometer with polystyrene calibration at 1601 cm^{-1}. Absorption bands are recorded in wave numbers (cm^{-1}). Pmr spectra were recorded in $CDCl_3$ solution with a Varian model T-60A instrument, operating at 60 MHz with a Nicolet, model TT-7, Fourier Transform attachment. Trimethylsilane was used as an internal standard and chemical shifts are reported in δ (ppm) units. Low resolution mass spectra were obtained with a Hitachi Perkin Elmer, model RMU-6D, single focusing spectrometer operating at 70 ev.

[3]E. Merck, Darmstadt, Germany.



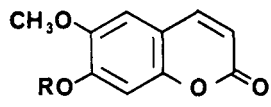


1 R = $COCH_3$
6 R = H

3 R = H
5 R = D-glucose

$CH_3(CH_2)_{24}COOH$

2



4 R = H
7 R = CH_3

0.001%), mp. 302–3°, $[\alpha]^{25}D$ −48.3 (c 0.2, $CHCl_3$), [Lit. (7) mp 298–300°, $[\alpha]^{25}D$ −49]; ir, νmax (KBr) 3500–3100, 2950, 1740, 1695, and 1250 cm^{-1}; nmr, ($CDCl_3$, 60 MHz) δ 5.52 (1H, dd *J* 6.6, 3.6 Hz, C_{15}-H), 4.45 (1H, m, w/2 20 Hz, 3α-H), 2.03 (3H, s, $OCOCH_3$), 1.25 (3H, s, CH_3), 0.92 (18H, s, 6 x CH_3); ms, *m/e* 498 (6%), 483 (4), 452 (3), 438 (9), 423 (8), 394 (3), 344 (9), 329 (6), 248 (35), 234 (100), 201 (18) and 189 (60). The structure assignment was confirmed by comparison (mmp and tlc) with an authentic sample.

Hydrolysis of **1** (20 mg) with 5% NaOH in 95% ethyl alcohol-water (5 ml) under reflux for 2 hr. afforded, after crystallization from *n*-hexane, aleuritolic acid (**6**, 17 mg), mp 303–304° d., [Lit. (7) 303–306° d.].

ISOLATION AND IDENTIFICATION OF HEXACOSANOIC ACID (**2**).—A sample (100 mg) of fraction 3 was purified on a silica gel PF 254 preparative plate, eluting with chloroform. Crystallization from *n*-hexane afforded a white powder (43 mg, 0.0002%) identified as hexacosanoic acid (**2**), mp 85–6° [Lit. (8) mp 88–89°]; ir, νmax (KBr) 3500–3000, 2900, 2650, 1700, 1470, 1280, and 940 cm^{-1}; nmr, ($CDCl_3$, 60 MHz), δ 2.24 (2H, t, *J*=6 Hz, C2-H_2), 1.25 (46H, broad m), and 0.95 (3H, t, *J*=5 Hz, C26-H_3); ms, *m/e* 396 (1%), 382 (2), 368 (9), 354 (6), 340 (6), 326 (1), 312 (5), 297 (1), 284 (3), 269 (1), 256 (5), 241 (1), 227 (1), 213 (2), 199 (1), 185 (5), 171 (4), 157 (3), 143 (3), 129 (12), 115 (4), 101 (6), 97 (14), 83 (17), 69 (30), 57 (55), 55 (60), and 43 (100). The physical and spectral data are in agreement with the structure hexacosanoic acid (**2**) for this isolate.

ISOLATION AND IDENTIFICATION OF β-SITOSTEROL (**3**).—Crystallization of fraction 12 (0.490 g) from hexane yielded β-sitosterol (**3**) as white crystals (460 mg, 0.002%), mp 132–3°, $[\alpha]^{26}D$ −36.6° ($CHCl_3$), [Lit. (8), mp 136–7°, $[\alpha]D$ −35°]. The structure was confirmed by comparison (mmp and tlc) with an authentic sample.

ISOLATION AND IDENTIFICATION OF SCOPOLETIN (**4**).—Fraction 43 (1.21 g) on crystallization from chloroform-petroleum ether (1:1) yielded yellowish green crystals of scopoletin (**4**) (83 mg, 0.0004%), mp 204–5° [Lit. (9), mp 203–4°]; uv, λmax (MeOH) 350 (log ε 4.04), 300 (3.45), 250 (3.43), 228 (4.11) and 210 nm (4.31); ir, νmax (KBr) 3300, 1715, 1610, and 1285 cm^{-1}; nmr, ($CDCl_3$, 60 MHz) δ 7.64 (1H, d, *J*=9.4 Hz, 4-H), 6.98 (1H, s, 8-H), 6.90 (1H, s, 5H), 6.30 (1H, d, *J*= 9.4 Hz, 3-H), 6.20 (1H, bs, removed by D_2O, 7-OH), 4.00 (3H, s, 6-OCH_3); ms, *m/e* 192 (M^+, 75%), 177 (M^+–15, 90), 164 (M^+–28, 55), 149 (M^+–28–15–28, 40), 107 (9), 105 (8) and 92 (12).

Methylation of a portion (15 mg) of the isolate with methyl iodide (0.2 ml) in dry acetone (5 ml) in the presence of K_2CO_3 (100 mg) at room temperature for 3 hours was followed by the usual work-up. Crystallization of the residue from *n*-hexane afforded 7-*O*-methylscopoletin

(7) as pale yellowish green crystals (10 mg), mp 142–3° [Lit. (8) 145°]; ms *m/e* 206 (M^+). These data established the isolate to be scopoletin (4).

ISOLATION AND IDENTIFICATION OF β-SITOSTEROL GLUCOSIDE (5).—Combined column fractions 85 and 86 (2.7 g) on crystallization from chloroform-petroleum ether (1:1) afforded β-sitosterol-β-D-glucoside (5) as a white powder (250 mg, 0.0012%), mp 297–8° d., $[\alpha]^{26}D$ −40.3° (pyridine) (Lit. (10) mp 290° d., (8), $[\alpha]D$ −41.5° (pyridine)]. The structure was confirmed by comparison (mmp, tlc) with an authentic sample.

BIOLOGICAL ACTIVITY.—Acetylaleuritolic acid (1) was inactive in the dose range 50–400 mg/kg in the P-388 lymphocytic leukemia in mice and also in the KB test system *in vitro*. Previous workers (7) have indicated that 1 was active in the P-388 system at doses of 1 mg/kg and 1.4 mg/kg. In agreement with the results reported by Williams and Cassady (11), scopoletin (NSC-405647) (4) was found to be inactive in the KB test system *in vitro*.

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