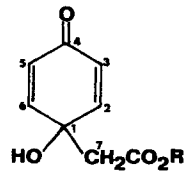
CYTOTOXIC AGENT FROM *SENECIO ANONYMUS* WOOD[1]

LESLIE T. GELBAUM, LEON H. ZALKOW* and DARRELL HAMILTON

School of Chemistry, Georgia Institute of Technology, Atlanta, Georgia 30332

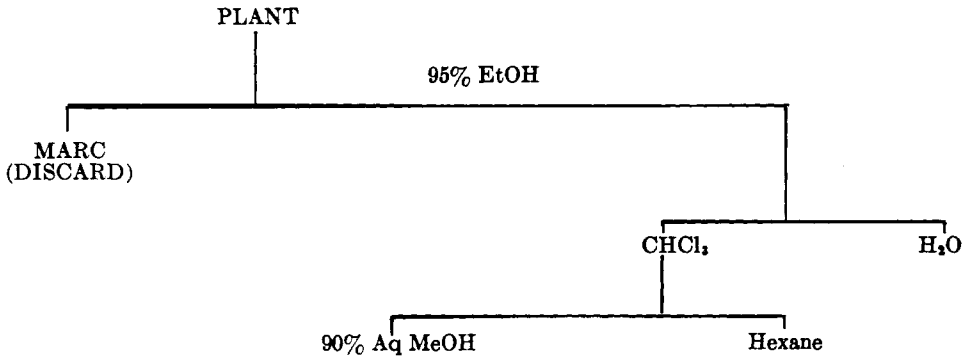
Senecio anonymus Wood is a common plant which grows abundantly along roadsides in the southeastern United States. Since it is known that the constituents of the genus *Senecio*, namely, pyrrolizidine alkaloids (1) and eremophilane sesquiterpenes (2) have cytotoxic effects, the 95% ethanol extract of the whole plant was screened in the P388 lymphocytic leukemia (PS) tumor system.[2] Results of this screen indicated activity in the extract.

In search of possible new sesquiterpenes, we chromatographed the hexane



1 R=CH_2CH_3

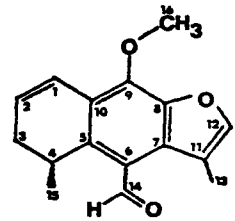
3 R=CH_3



Scheme I

The 95% ethanol extract was then partitioned as indicated in scheme I. PS tumor screens of each fraction indicated that the antitumor activity was concentrated in the 90% aqueous methanol fraction. Chromatography led to the isolation of a white crystalline material of mp 67–69°. This was identified as ethyl-1-hydroxy-4-oxo-2,5-cyclohexadiene-1-acetate (1) (jacaranone ethyl ester), on the basis of its ^1H and ^{13}C nmr spectra.

fraction and isolated a colorless oil and a crystalline material of mp 92–94°. The oil was identified as ethyl oleate and the solid as 14-oxo-1,2-dehydrocacalol methyl ether (2). The structures of these compounds were determined from their spectroscopic and physical properties.



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[1] *Senecio anonymus* Wood was formerly known as *Senecio smallii*, *Thodora*, **75**, 211–219 (1973) and *N. Am. Flora* II, **10**, 68 (1978).

[2] The screening of the plant extracts was carried out under the auspices of the National Cancer Institute (NCI).

EXPERIMENTAL[3]

PLANT MATERIAL.—*Senecio anonymus* Wood was collected in July 1977 near Barnesville, Georgia, and was identified by Dr. T. M. Barkley at the Kansas State University where a herbarium sample has been deposited.

EXTRACTION.—Air dried whole plant material (3 kg) was mascertaed in a blender with 95% ethanol and continuously extracted for 48 h. The ethanol was removed in vacuo leaving 864g of crude extract. This fraction had a T/C of 133 in the PS tumor screen. The crude extract (420g) was partitioned between chloroform (2 liters) and water (2 liters). The residue from the chloroform partition was then partitioned between hexane (0.8 liter) and 10% aqeous methanol (0.8 liter). Removal of the hexane and aqueous methanol left 59.3g and 54.1g, respectively. PS tumor and KB screens indicated that the antitumor activity was concentrated in the aqueous methanol fraction with a T/C of 138 and an ED_{50} of 0.20 μg/ml, respectively.

ISOLATION AND CHARACTERIZATION OF JACARANONE ETHYL ESTER (1).—The aqueous methanol extract was dissolved in ether and extracted with 5% aqueous NaOH. Tlc of the ether soluble residue on silica gel with benzene-ether (1:1) indicated one major component (R_f=0.37). Chromatography of 4.6g of the ether soluble material on 300g of silica gel (100–200 mesh) with benzene-ether (1:1) as the solvent gave 0.47g of a viscous oil that was crystallized from ether-hexane to give colorlesss crystals: mp 67–69°, lit (3) 71°; ^{1}H nmr ($CDCl_3$) δ 1.17 (3H, t, J=7 Hz, C-10 H), 2.67 (2H, s, C-7 H), 4.12 (2H, q, J=7 Hz, C-9 H), 4.65 (1H, bs, OH), 6.00 (2H, d, J=10 Hz, C-3, 5 H), 6.90 (2H, d, J=10 Hz, C-2, 6 H); ^{13}C nmr ($CDCl_3$) 12.5 (q, C-10), 42.9 (t, C-9), 59.2 (t, C-7), 65.5 (s, C-1), 125.5 (d, C-3, 5), 148.3 (d, C-2, 6), 167.0 (s, C-8), 183.0 (s, C-4); ir, ν $CHCl_3$ 3450, 1730, 1675 cm^{-1}; UV, λ max (CH_3OH) 227 (ϵ 9,333); ms exact mass: found 196.0729, calculated for $C_{10}H_{12}O_4$ 196.0736, M^+ 196 (6.3%), 150 (34), 122 (32), 109 (100), 108 (34), 107 (30), 88 (75).

ISOLATION AND CHARACTERIZATION OF ETHYL OLEATE AND 14-OXO-1,2-DEHYDROCACALOL METHYL ETHER (2).—The crude hexane fraction (20g) was chromatographed on 200 g of acid-washed alumina (100–200 mesh). The column was eluted with benzene-ether mixtures starting with 100% benzene; the ether was increased to 1:1. Fraction 2 from this chromatography, which was eluted with 100% benzene, was then chromatographed on an E.M. Reagents prepack column (size C) containing silica gel 60. Fifty 60 ml fractions were taken; hexane ether (9:1) was the eluting solvent. The third fraction yielded a colorless oil which had a ^{1}H nmr, mass spectrum and gc retention time identical to an authentic sample of ethyl oleate. Fractions 22–24 crystallized spontaneously on slow evaporation of the solvent yielding colorless crystals (0.11g): mp 92–94°; ^{1}H nmr δ 1.17 (3H, d, J=7.0 Hz, C-15 H), 2.30 (2H, m, C-3 H), 2.37 (3H, d, J=1.2 Hz, C-13 H), 4.07 (1H, m, C-4 H), 4.26 (3H, s, C-16 H), 5.99 (1H, d, d, d, d, $J_{1,2}$=9.8 Hz, $J_{2,3\alpha}$=6.1 Hz, $J_{2,3\beta}$=2.7 Hz; $J_{2,4\alpha}$=0.7 Hz, C-2 H), 6.95 (1H, d, d, $J_{1,2}$=9.8 Hz, $J_{1,3\beta}$=2.7 Hz, C-1 H), 7.36 (1H, q, J=1.2 Hz, C-12 H), 10.7 (1H, s, C-14 H); ir, ν $CHCl_3$ 2930, 2860, 1675, 1600, 1550, cm^{-1}; uv, λ max (CH_3OH) 268 (ϵ=2.06 x 10^4), 276 (ϵ=2.15 x 10^4), 302 (ϵ=1.18 x 10^4); $[\alpha]_{589}^{22}$+79.6 (C=2.26, chloroform); ms exact mass found 256.1040, calculated for $C_{16}H_{16}O_3$ 256.1100, M+ 256 (68%), 241 (100), 198 (15), 141 (16), 115 (118).

DISCUSSION

The structure of **1** can be unequivocally deduced from its spectroscopic properties. Its ^{1}H and ^{13}C nmr very closely resemble the spectra of methyl-1-hydroxy-4-oxo-2,5-cyclohexadiene-1-acetate, jacaranone (**3**), which was isolated from *Jacaranda caucana* (4). Both the methyl ester **3** and the ethyl ester **1** have been isolated from other Senecio species (5). The extraction procedure used in the previous isolation of these compounds did not include any ethanol, so the ethyl ester is most likely present in the plant and not an antifact produced by the extraction procedure.

Jacaranone was found to have significant antitumor activity in both the KB(ED_{50}=2.1 μg/μl) and PS tumor (T/C 165) screens (4). Compound **1**, the ethyl ester of jacaranone, has been tested in a KB screen and also found to have significant activity with an ED_{50} of 3.3 μg/μl. It is therefore believed that **1** is the active constituent in the plant material. Compound **1** is now undergoing *in vivo* testing.

The structure of **2** was also determined from its spectroscopic properties These were consistent with those

[3]Mp's were taken on a Thomas-Kofler micro hot stage model 651 and are uncorrected. Ir spectra were recorded with a Perkin-Elmer 237B spectrometer. ^{1}H nmr spectra were obtained with a Varian T60 or JEOL-PFT-100 FT spectrometer with Me_4Si as an internal standard (δ0); ^{13}C nmr spectra were run on the JEOL instrument. Mass spectra were obtained on a Hitachi Perkin-Elmer Model RMU-7L or a Varian model 112S interfaced to an SS200 data system. Gas chromatography was carried out on a Varian 2700 gas chromatograph with an OV 101 fused silica capillary column at 190°.

previously reported for its isolation from *Senecio othonnae* Bieb (5). This structure was also confirmed by a single crystal x-ray analysis which will be published elsewhere. *S. othonnae* Bieb. is also reported to contain both jacaranone methyl and ethyl ester (5).

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