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15-DEACETYLSERGEOLIDE, A POTENT ANTILEUKEMIC QUASSINOID FROM *PICROLEMMA PSEUDOCOFFEA*[1]

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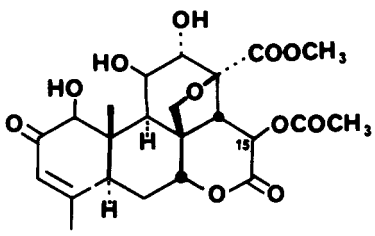
ABSTRACT.—Investigation of the leaf extract of *Picrolemma pseudocoffea* afforded a new antineoplastic quassinoid, 15-deacetylsergeolide (**3**), and two known quassinoids, isobruceine B (**1**) and sergeolide (**2**). The structure of **3**, which displayed strong antileukemic activity in the P-388 test system, was established through the interpretation of spectral data and chemical correlation to **2**.

The quassinoids (2), the bitter principles isolated from the Simaroubaceous plants, form a group of degraded triterpenes some of which display diverse biological activities ranging from antitumor (3), antiviral (4), antimalarial (5) to amebicidal (6) and antifeedant properties (7). Recently, these quassinoids have also attracted much attention as synthetic target molecules (8,9).

Previous studies (10) of the roots and stems of the French Guianan Simaroubaceae *Picrolemma pseudocoffea* Ducke resulted in the isolation and structural elucidation, along with the known isobruceine B (**1**) (11), of a highly cytotoxic quassinoid, sergeolide (**2**). Investigation of the leaf extract of *P. pseudocoffea* has now led to the isolation of a new antileukemic quassinoid that has been identified as 15-deacetylsergeolide (**3**).



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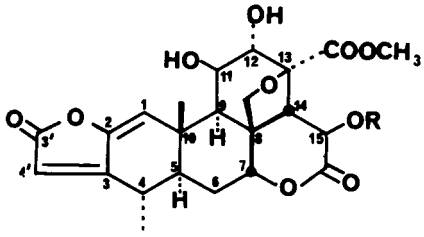

2 R=$COCH_3$
3 R=H

The molecular formula for **3**, mp 295-300° (dec.), was found by hrms to be $C_{23}H_{26}O_{10}$, and thus the molecular weight of **3** is 42 a.m.u. lower than that of sergeolide (**2**). The ir spectrum (nujol) showed carbonyl absorptions at 1730 (δ-lactone), 1758 (ester), and 1782 cm^{-1} (sh) (butenolide ring). The uv spectrum of **3** (λ max 278 nm, ε 26.514) indicated that **3** possesses, like sergeolide (**2**), a γ,δ-unsaturated butenolide ring. The 400 MHz pmr spectrum of 15-deacetylsergeolide (**3**), which is devoid of a signal due to an acetoxy group, is presented in Figure 1 (see Experimental section). The structural similarity between the quassinoids **2** and **3** was supported by the near identity of the chemical shifts and multiplicities of all hydrogen atoms except for H-14 and H-15. A significant upfield shift was observed for the latter which gave rise to a well-defined doublet at 5.16 ppm (J=13 Hz). The chemical shift of this resonance indicates that **3** does not possess an ester group at C-15 and is consequently 15-deacetylsergeolide.

Finally, the structure of **3** was confirmed by chemical correlation with **2**. Acetyla-

[1]For previous paper in this series, see Bhatnagar *et al.* (1).

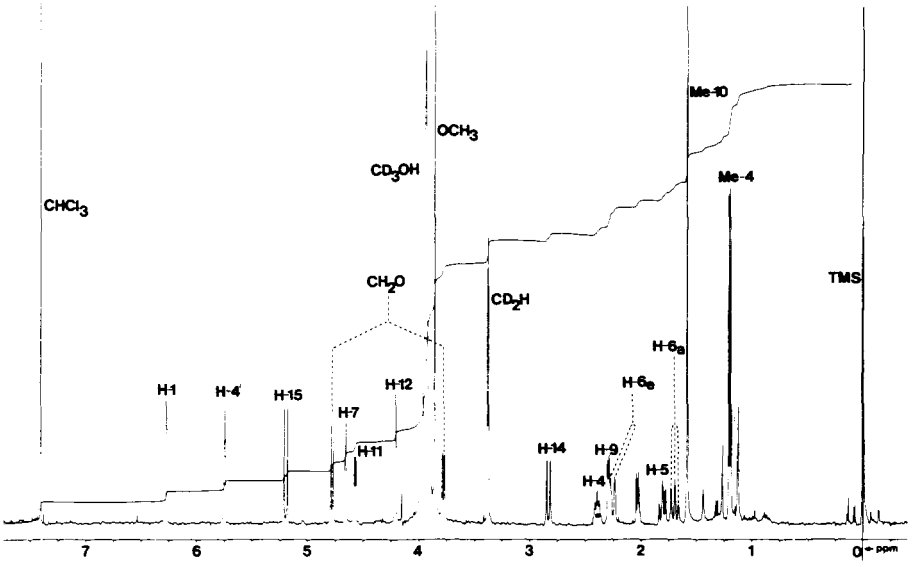

FIGURE 1. 400 MHz ^{1}H-nmr spectrum of 15-deacetylsergeolide (**3**) in $CDCl_3$ containing 30% MeOH-d_4.

tion of **3** (Ac_2O-pyridine) under controlled conditions led, in good yield, to **2** (identity: mp, tlc, ms, 400 MHz pmr).

Compound **3** does not seem to be an artifact from **2** during the aqueous isolation procedure since an identical extraction of the root and stems afforded only **1** and **2** (10).

In the murine P-388 lymphocytic leukemia system, deacetylsergeolide (**3**), which seems to be less toxic than sergeolide (**2**), displayed potent in vivo activity; e.g., at 0.63 and 1.25 mg/kg it afforded T/C values of 150 and 169, respectively.[2] With the P-388 in vitro system (12), **3** exhibited only moderate cell growth inhibition (ED_{50} 8.4×10^{0}).

Anticancer activity in the quassinoids is well documented, and the structural requirements for this activity in the P-388 lymphocytic leukemia system are well established (13,14). Thus, it is well known that A-ring oxygenation and unsaturation, an oxygen linkage from C-30 to C-11 or C-13, a free hydroxyl group in ring A and at C-12, and an ester at C-6 or C-15 are all required for in vivo activity. It is interesting to note that the butenolide function in **3** in some way compensates the lack of the requisite A-ring oxygenation and the C-15 or C-6 ester chain.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Kofler melting point apparatus and are uncorrected. Optical rotations were determined (room temperature) with a Rousel-Jouan Quick Polarimeter. Ir spectra were recorded in nujol on a Perkin-Elmer model 257, and the uv spectra were measured in EtOH with a spectrometer Duospac 203 (Jobin-Yvon). Electron impact mass spectral determinations were performed with AEI model MS-50. The 400 MHz ^{1}H-nmr were recorded with a Bruker WM-400 spectrometer.

All solvents employed for chromatography were redistilled. Analytical tlc was carried out on plasticized plates coated with silica gel F 1500 LS 254 (Schleicher & Schüll) or 60 F 254 (Merck). Column chromatography was performed with silicic acid (Mallinkrodt) and celite. H_2SO_4 spray (gives a deep red with some quassinoids) followed by heating (10 min) easily developed the quassinoids.

EXTRACTION AND ISOLATION OF 15-DEACETYLSERGEOLIDE (**3**).—The plant material, collected in

[2]These data are the results of screening performed under the auspices of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

1981 in French Guiana, consisted of the leaves of *P. pseudocoffea* Ducke. A voucher specimen (No. Moretti 1292) is deposited in the herbarium of the Museum d'Histoire Naturelle de Paris. The dried, ground leaves (320 g) were defatted by percolation with hexane at room temperature. The mass was then stirred for several hours with hot H_2O (70-75°), separated by filtration in vacuo and resuspended in fresh hot H_2O. This was repeated several times until the filtrate was no longer bitter. The combined aqueous extracts were concentrated in vacuo and then continuously extracted with $CHCl_3$. Evaporation of the solvent yielded a brown foam (2.2 g) that was chromatographed on silicic acid-celite (3:1) (120 g); CH_2Cl_2 containing increasing amounts (1 to 5%) of MeOH was the eluent. Elution with CH_2Cl_2 containing 2% MeOH afforded a mixture (530 mg) of isobruceine B (**1**) and sergeolide (**2**) and elution with CH_2Cl_2 containing 3% MeOH gave 15-deacetylsergeolide (**3**) (92 mg).

Recrystallization of **3** from a mixture of $CHCl_3$ and MeOH afforded colorless prisms, mp 295-300° (dec.); $[\alpha]^{24}D$ −145° (*c*, 1.0 in pyridine). Hrms $M^{+\cdot}$ at *m/z* 462.1522; required for $C_{23}H_{26}O_{10}$: 462.1526; 400 MHz ^{1}H-nmr spectrum (Figure 1) ($CDCl_3$+30% CD_3OD) δ 1.18 (3H, d, *J*=7 Hz, Me-4), 1.57 (3H, s, Me-10), 1.69 (1H, ddd, *J*=15; 2.5; 2.5 Hz, H-6a), 1.80 (1H, ddd, *J*=13; 2; 9 Hz, H-5), 2.24 (1H, ddd, *J*=15; 2; 2 Hz, H-6e), 2.28 (1H, d, *J*=5 Hz, H-9), 2.38 (1H, m, H-4), 2.81 (dd, *J*=13; 2 Hz, H-14), 3.76 (1H, dd, *J*=8; 1 Hz), 4.75 (1H, d; *J*=8 Hz) (AB, -CH_2O-), 3.83 (3H, s, OCH_3), 4.17 (br. s, H-12), 4.54 (1H, br d, *J*=5 Hz, H-11), 4.63 (1H, br s, H-7), 5.16 (1H, d, *J*=13 Hz, H-15), 5.72 (1H, br s, H-4'), 6.23 (1H, br s, H-1).

CONVERSION OF **3** INTO **2**.—Compound **3** (10 mg) was treated with Ac_2O-pyridine (1:1) (2 ml). The mixture was kept at room temperature for 1 h. Usual work-up gave **2** (10 mg), homogeneous on tlc ($CHCl_3$-10% MeOH), which was purified by recrystallization from Me_2CO.

ACKNOWLEDGMENTS

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