Journal of Natural Products
Vol. 53, No. 4, pp. 968-971, Jul-Aug 1990

ELABUNIN, A NEW CYTOTOXIC TRITERPENE FROM AN EAST AFRICAN MEDICINAL PLANT, *ELAEODENDRON BUCHANANII*

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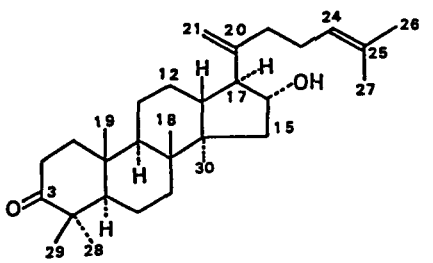
ABSTRACT.—A chemical investigation of biologically active compounds from an East African medicinal plant, *Elaeodendron buchananii*, has led to the isolation and characterization of a new dammarane triterpene, elabunin [**1**], which exhibits moderate cytotoxic activity (ED_{50} 1 μg/ml) against L-1210 leukemic cells.

In East Africa, the root bark of *Elaeodendron buchananii* Loes (Celastraceae) is used as a folk medicine to cure wounds, syphilis, and diarrhea (1). In our continuing study of biologically active natural products from tropical plants, we found that the MeOH extract of the root bark of *E. buchananii* exhibited various biological activities, such as insecticidal, molluscicidal, antimicrobial, and cytotoxic. This paper describes the isolation and characterization of a new cytotoxic dammarane triterpene.

The MeOH extract of the root bark of *E. buchananii* showed cytotoxic activity (ED_{50} 100 μg/ml) against L-1210 leukemic cells in preliminary routine screening. The isolation of the bioactive principle was guided by the cytotoxicity assay and led to the isolation of a new dammarane-type triterpene, for which the name elabunin [**1**] was proposed. Lupeol was also isolated from the active fraction, although it did not exhibit cytotoxicity.

Elabunin [**1**] gave the molecular formula $C_{30}H_{48}O_2$ by hrms analysis (*m/z* 440.3652). Ir in $CHCl_3$ showed absorptions at 3200 to 3600 cm^{-1} (hydroxyl), at 1705 cm^{-1} (carbonyl), and at 880 cm^{-1} (*exo*-methylene group).



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The ^{1}H-nmr spectrum of **1** showed some signals typical of the dammarane type triterpenes, such as five singlet methyl signals at δ 0.9–1.1 (methyls on quaternary carbons). In addition, two methyl signals were observed at δ 1.6 and δ 1.7 (vinyl methyls), and one methine proton on a carbon having a hydroxy at δ 4.05, two *exo*-methylene protons at δ 4.3, and a vinyl proton at 5.14 ppm were observed. The ^{13}C-nmr data showed the presence of seven methyl, nine methylene, five methine, and one hydroxylated carbon. This indicated elabunin [**1**] to be a dammarane derivative containing the following functions: one ketone, one hydroxy, and two double bonds. The ^{13}C-nmr data of elabunin were compared with those reported for dammara-20,24-dien-3-ol (2) including four olefinic carbons on the side chain, and for 20-hydroxydammar-24-en-3-one (3) containing one carbonyl. This comparison suggested that elabunin could be a dammara-20,24-dien-3-one with one hydroxyl. The location of the hydroxyl group could be assigned to C-16 through the application of 2D homonuclear ^{1}H-^{1}H correlation spectroscopy (^{1}H-^{1}H COSY) and a ^{1}H-^{1}H decoupling experiment as follows.

In the ^{1}H-^{1}H COSY of elabunin [**1**], the signal at δ 4.05 ppm assigned to the proton on a hydroxylated carbon was ob-

served to be coupled with three protons resonating as double doublets at δ 2.25 (H-17), δ 2.08 (H-15a), and δ 1.15 ppm (H-15b). A decoupling experiment with selective irradiation at δ 4.05 collapsed these 3 signals into doublets (Figure 1). This observation established the presence of the partial structure shown in Figure 2. Incorporation of this partial structure into the dammarane skeleton is possible only by locating the hydroxy group at C-16. The configuration of the 16-OH could be α as indicated by the coupling constant.

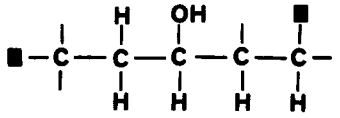


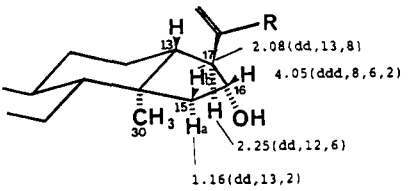
FIGURE 1. Partial structure of elabunin.

The absolute stereochemistry of elabunin [**1**] was determined by application of ^{1}H-^{1}H COSY, 2D homonuclear ^{1}H-^{1}H nOe correlation spectroscopy (^{1}H-^{1}H NOESY), nOe difference spectroscopy, and cd spectroscopy. In the ^{1}H-^{1}H NOESY spectrum, the C-30 methyl signal, appearing downfield at δ 1.12 ppm due to the effect of the 16-OH group, is shown to be related to H-17 at δ 2.25 ppm by the presence of cross peaks. Also the C-18 methyl was related to H-13 and H-15b and the C-19 methyl was related to H-2ax.

These observations provided evidence for the close spatial orientation of C-30 methyl, the 16-OH, and H-17. The analysis of the ^{1}H-^{1}H NOESY experiment led to the structure proposed for the D ring of elabunin.

For the side chain, the nOe difference spectra also provided important information. Thus, irradiation at the H-26, readily distinguishable from that at H-27 by a long-range coupling with H-24, gave an nOe to H-16 (6.24%) and H-24 (9.03%). On the other hand, irradiation at δ 1.63 (H-27) gave an nOe to H-16 (4.55%). Consequently, the side chain was established as shown in Figure 3.

In order to define the absolute structure, the cd spectrum was measured in MeOH. The cd spectrum showed a posi-



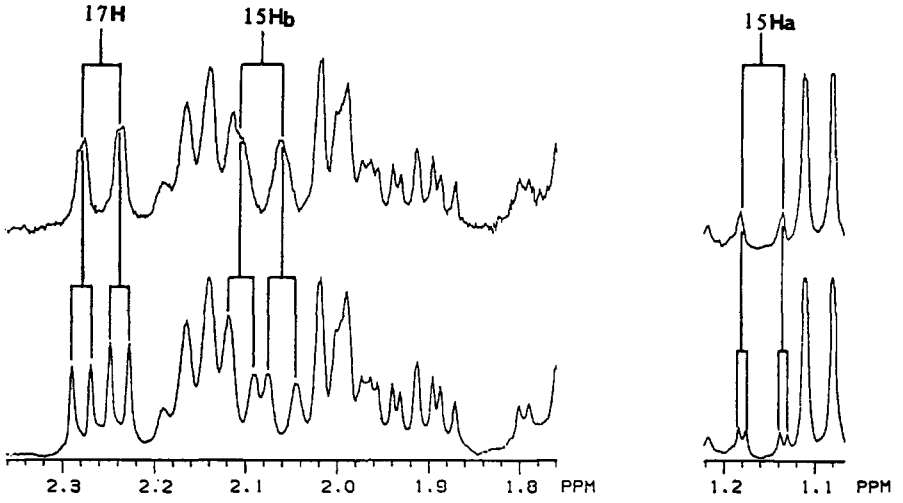


FIGURE 2. Spin-decoupling ^{1}H nmr (irradiated at 4.05 ppm).

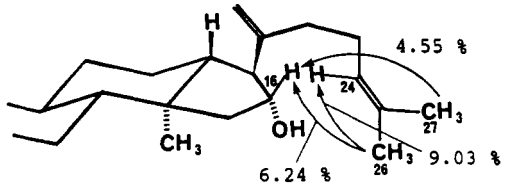


FIGURE 3. NOe difference spectroscopy.

tive Cotton effect at 286 nm. All of the spectral data concluded that elabunin is 16α-hydroxydammara-20,24-dien-3-one [**1**].

In general, it has been known that dammarane-type and lupane-type triterpenes could be biosynthesized from the dammarane cation. Both elabunin and lupeol isolated from the same root bark of *E. buchananii* may be biosynthesized through this pathway. There are many reports of naturally occurring dammarane compounds (4–6). However, a dammarane structure with a 16-OH is a rarity. Elabunin exhibited moderate cytotoxicity (ED_{50} 1 μg/ml) against L-1210 leukemic cells.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points (mp) were determined with a Sybron Thermolyne Mp-12615 and were uncorrected. Ir spectra were measured by a Perkin-Elmer 1310 Infrared Spectrophotometer. Uv spectra were taken by a Hitachi 100-80 Spectrophotometer. ^{1}H- and ^{13}C-nmr data were recorded on a JEOL GX-400 instrument. TMS was used as internal reference for nmr measurements. The high resolution ms spectrum was obtained with a JEOL DX-300 (di-ei, 70 eV). The cd spectrum was measured with a Jasco J-40 spectropolarimeter.

PREPARATION OF PLANT MATERIAL.—The root bark of *E. buchananii* was collected near Monbasa, Kenya, and air-dried. The plant was identified by Geoffrey M. Mungai of the East African Herbarium Nairobi, Kenya, where a voucher specimen is kept. The pulverized sample (1050 g) was extracted with MeOH at room temperature, and the MeOH was evaporated in vacuo under 40° to give a brown tar (115.6 g). The residue was was partitioned into *n*-hexane-, $CHCl_3$-, EtOAc-, and H_2O-soluble fractions. The biologically ac-, and H_2O-soluble fractions. The biologically active *n*-hexane fraction (2.2 g) was chromatographed on Si gel eluting with *n*-hexane followed with *n*-hexane containing increasing amounts of EtOAc. The active fraction was rechromatographed on Sephadex LH-20, followed by chromatography on Si gel, to yield a new dammarane triterpene (50 mg) for which the name elabunin [**1**] was proposed. In addition, lupeol (10 mg) and several other triterpenes were also isolated from the active fraction; however, none of them exhibited cytotoxicity.

LUPEOL.—Lupeol was identified by comparison with ^{1}H- and ^{13}C-nmr and ms data of an authentic sample.

ELABUNIN [**1**].—$C_{30}H_{48}O_2$ (by hrms *m/z* 440.3652, calcd 440.3654); mp 108–109°; uv (EtOH) 280 (ε 200); cd (MeOH) 286 nm ($\Delta\epsilon + 0.6$); ir ($CHCl_3$) 3200–3600 (hydroxyl), 1705 (carbonyl), 880 cm^{-1} (*exo*-methylene); ^{1}H nmr ($CHCl_3$) δ 0.93, 0.99, 1.03, 1.08, 1.11 (15H, s, -Me), 1.16 (1H, bd, J = 13 Hz, Ha-15), 1.62, 1.69 (6H, s, -Me), 2.08 (1H, bd, J = 13 Hz, Hb-15), 2.25 (1H, dd, J = 12 and 6 Hz, H-17), 4.05 (1H, ddd, J = 8, 6, and 2 Hz, H-16), 4.83 (2H, s, C=CH_2), 5.14 (1H, bt, J = 6.9 Hz, CH=C); ^{13}C nmr 15.69 (q, C-19), 15.90 (q, C-18), 17.54 (q, C-30), 17.71 (q, C-27), 19.61 (t, C-6), 20.98 (q, C-29), 21.74 (t, C-11), 24.35 (t, C-23), 25.66 (q, C-26), 26.66 (t, C-12), 26.75 (q, C-28), 34.03 (t, C-2), 34.15 (t, C-15), 34.59 (t, C-7), 36.90 (s, C-10), 39.76 (t, C-1), 40.14 (s, C-8), 41.72 (t, C-22), 44.97 (d, C-13), 47.34 (s, C-4), 48.16 (s, C-14), 49.80 (d, C-9), 55.27 (d, C-5), 58.34 (d, C-17), 77.64 (d, C-16), 109.59 (t, C-21), 124.15 (d, C-24), 131.67 (s, C-25), 149.75 (s, C-20), 217.87 (s, C-3) ppm.

ACKNOWLEDGMENTS

We are grateful to Mr. H. Naoki for nmr and cd measurements, to Dr. S. Kurozumi for cytotoxicity assays, and to Mr. A. Chapya for the plant collection.

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Received 3 October 1989