ISOLATION OF *ent*-LABDANE (+)-12,15-EPOXYLABDA-8(17), 12,14-TRIEN-16-YL ACETATE FROM THE SEEDS OF *Turraeanthus africanus* AND ITS CYTOSTATIC/CYTOTOXIC EFFECT ON THE GROWTH OF CANCER CELLS *in vitro*

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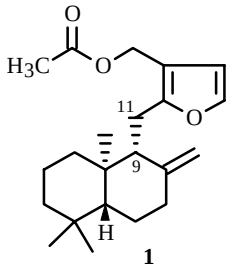
(+)-12,15-Epoxylabda-8(17),12,14-trien-16-yl acetate has been isolated from the seed kernels of Turraeanthus africanus *(Nelw. ex DC). The stereo structure was confirmed by 1D- and 2D-NMR spectroscopy. Cytostatic/cytotoxic test of (I) on the growth of cancer cells* in vitro *gave positive results.*

Key words: Cytostatic, cytotoxic, *Turraeanthus africanus*, limonoid, *Meliaceae*.

Turraeanthus africanus (Nelw. ex DC) is a plant native to West Africa [1, 2] and belongs to the Meliaceae family. It is a medium-sized tree up to 110 ft high and 4 ft in diameter. The wood bark and the leaves are used for poisoning fish and its oil is also employed in folk medicine as an abortive and fish poison. Work done on the Meliaceae shows that they contain bitter active principles called "Limonoids," which are tetranortriterpenoid compounds that have several biological activities including antifeedancy against insects [3]. Previous studies on this plant afforded the protolimonoids turraeanthin and epi-turraeaenthin (melianol acetate) from the heartwood [3]. A systematic phytochemical investigation on the seed kernels of *T. africanus* resulted in the isolation of **1**.

T. G. Halsall reported this labdane diterpene derivative in 1974 at a symposium in Ottawa (Canada) [4] from a nonspecified part of *T. africanus.* Cambie et al synthesised **1** starting with sclareol and manoyl oxide, both of known absolute configuration. Their products however, were the (–)-isomers. This is supported by the optical rotations of $[\alpha]_D^{20}$ –18° (c, 2.3, $CHCl_3$) for **1** (synthetic) compared with $[\alpha]_D^{20}$ +21° (conc. unknown) for the natural **1** isolated by Halsall [4].



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Compound **1** was obtained as a white jelly like substance after rechromatography by preparative TLC (silica gel, $CHCl_3$–MeOH, 4:1) on the petroleum ether/ethyl acetate fraction. It gave an orange to violet coloration on a TLC (silica gel) plate when sprayed with 2% 4-dimethylaminobenzaldehyde in ethanol (Erhlich's reagent) and developed in a hydrogen chloride chamber (Maier and Grant) [5]. This was evidence for **1** as a limonoid. Yield of the product was 0.75 g; 0.17% rel. to the crude extract, $[\alpha]_D^{26.6}$ + 13.8° (1.0 mg/mL, $CHCl_3$). Spectral data, which are in good agreement with those obtained by Cambie [5], are summarized as follows: IR (ν_{max}, cm^{-1}): 3100 (furan), 1743.5 (ester), 1643, and 1514 (aromatic C=C), 893 (C=CH_2), 752 (furan).

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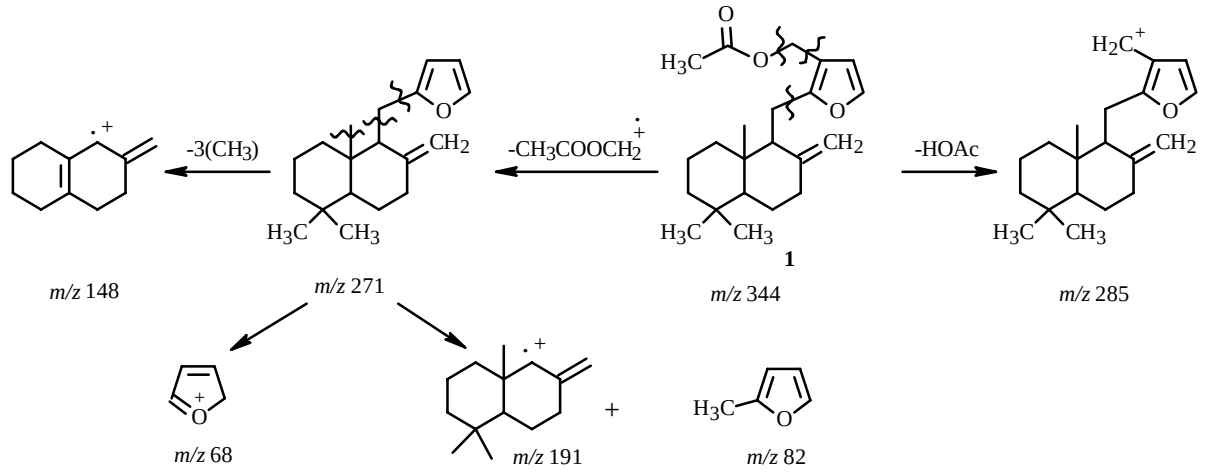

Fig. 1. Fragmentation pattern of the proposed structure of **1**.

^{1}H NMR (400 MHz, $CDCl_3$, J/Hz): 1.13, 1.17 (2H, H-1, H-1), 1.48 (2H, m, H-2), 1.18, 1.38 (2H, H-3), 1.17 (1H, t, H-5), 1.31, 1.71 (2H, q, H-6), 1.99, 2.33 (2H, H-7), 2.30 (1H, s, H-9), 2.73, 2.78 (2H, d, H-11), 6.28 (1H, d, J = 2.02, H-14), 7.20 (1H, d, J = 1.77, H-15), 4.91 (2H, s, H-16), 4.53, 4.73 (2H, d, J = 1.27, J = 1.52, H-17), 2.04 (3H, s, H-22), 0.80 (3H, s, H-18), 0.86 (3H, s, H-19), 0.74 (3H, s, H-20).

^{13}C NMR (100 MHz, $CDCl_3$): 38.9 (CH_2, t, C-1), 19.3 (CH_2, t, C-2), 42.0 (CH_2, t, C-3), 33.5 (s, C-4), 55.1 (CH, d, C-5), 24.1 (CH_2, t, C-6), 38.0 (CH_2, t, C-7) 148.4 (s, C-8), 54.1 (C-9), 39.6 (s, C-10), 21.6 (CH, d, C-11), 154.5 (s, C-12), 113.7 (s, C-13), 111.5 (CH, d, C-14), 140.2 (CH, d, C-15), 58.1 (CH_2, t, C-16), 106.9 (CH_2, t, C-17), 21.8 (CH_3, q, C-18), 33.6 (CH_3, q, C-19), 14.2 (CH_3, q, C-20), 171.1 (s, C=O), 21.6 (CH_3, q, $COOCH_3$).

EIMS: mz (%): 344 (< 1%), 284 (M-HOAc) (100), 269 (20), 191 (12), 137 (24).

The proposed structure of **1** was ultimately confirmed by its fragmentation pattern as shown in Fig. 1.

Our experiments show that H-5 is close enough to interact with C-19 methyl protons. Therefore both the C-18 methyl and H-5 must be on the same side of the labdane nucleus. H-11 and H-5 have no such interaction with C-9, C-18, and C-20 methyl groups, thereby establishing the α stereochemistry of C-9, C-20, and C-19 methyl groups.

The absolute configuration and the stereochemistry at the four asymmetric centers (C-4, C-5, C-9 and C-10) were therefore established and confirmed as suggested by R. C. Cambie et al. [5]. The synthesised compound obtained by Cambie et al. from sclareol [5] has optical rotation of –18° (c 2.3, $CHCl_3$), which is the only parameter different from what was obtained in the present investigation.

The concentration effects of **1** G150 (the concentration which resulted in a half maximal retardation of cell growth and TGI (the concentration which resulted in complete retardation of cell growth), were determined on the following tumor cell lines: HMO2, HepG2 liver carcinoma, and MCF7 (mammary gland carcinoma). From the curve of the concentration effect the following results were obtained.

HMO2: GI50-value =5.5; TGI-value = 8.8

HepG2: GI50-value =6.6; TGI-value = >10. (90% retardation with 10 μm/mL).

MCF7: GI50-value =5.6; TGI-value = 9.7.

The results were very moderate and significant. In all cases total retardation was achieved as the concentration effect increases. If a 100% retardation of the cell growth could not be achieved, then the retardation effect of the highest test concentration, i.e., 10 μm/mL, was given.

EXPERIMENTAL

Plant Material. The seed kernels of *T. africanus* were collected from the Kakum Game Reserved, Cape Coast in the Central Region of Ghana. Mr. Agyakwah of the Botany Department, University of Cape Coast, identified them.

Extraction and Isolation. The seed kernels of *T. africanus* (541.0 g) were thoroughly cleaned and dried in the oven for 24 hours at 40°C, after which they were ground into fine powder and extracted with a MeOH–CH_2Cl_2 (1:1) mixture (2.5 liters) by shaking at room temperature for 5 days. The extract was filtered and solvent removed under reduced pressure to give a brown residue (14.7 g). This was column chromatographed on silica gel in petroleum ether–ethyl acetate to give an impure compound (1.2 g) which was further purified by preparative TLC (silica gel, $CHCl_3$–MeOH, 4:1) to give a white jelly-like compound identified to be the epoxide labdane (**1**), R_f 0.75, (ethyl acetate).

Bioassay. Compound **1** was tested for its cytostatic/cytotoxic effect on the growth of cancer cells *in vitro*.

The tests were done according to the N.C.I guidelines with the following tumor cell lines; HMO2, HepG2 liver carcinoma and MCF7 (mammary gland carcinoma). The cells were cultivated on 96 – well microtiter plates. The medium was RPMI 1640 with 10% fetal lamb serum. Twenty-four hours after seeding, the test samples (concentration in solution: 0.1, 0.5, 0.15, and 10.0 μg/mL) were added and the cells cultivated for a further 48 hrs. After this time the cell numbers (protein determination with sulforhodamine) were determined. The test samples were dissolved in MeOH, whose concentration in the test solution was 0.1%. From the curve of the concentration effect the following values for **1** were determined:

GI50 = conc. which resulted in a half maximum retardation of cell growth.

TGI = conc. which resulted in complete retardation of cell growth.

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