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KAURANE AND KAURENE DITERPENES FROM THE STEM BARK OF *XYLOPIA ACUTIFLORA**

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Key Word Index—*Xylopia acutiflora*; Annonaceae; 16α-hydroxy-(−)-kaur-16-en-19-oic acids.

Abstract—Four diterpenes were isolated from the stem bark of *Xylopia acutiflora* and characterized as (−)-kauran-16α-ol, 7β-acetoxy-(−)-kaur-16-en-19-oic acid, 15-oxo-(−)-kaur-16-en-19-oic acid, and 16α-hydroxy-(−)-kauran-19-oic acid.

Xylopia acutiflora (Dunal) A. Rich. (Annonaceae) is a shrub or small tree of the evergreen forests of west Africa [1]. Two allied sympatric species, *X. aethiopica* A. Rich. and *X. quintasii* Engl. et Diels, have been reported to yield kaurane, trachylobane and kolavane diterpenes [2–5]. In this paper we report the isolation of four diterpenes of the kaurane type from a sample of stem bark of *X. acutiflora* (voucher: D. W. Thomas 595, at Kew) collected in the Korup Forest Reserve, Cameroun, in 1979.

On standing the concentrated petrol (bp 40–60°) extract yielded a novel diterpene dimer which is still under investigation. CC of the supernatant over Si gel gave more dimer and four diterpenes (A–D).

Diterpene A (yield 0.2%) analysed for $C_{20}H_{34}O$. Mp (210–216°), $[\alpha]_D^{21}$ (−40°), and spectral data (IR, ^{1}H NMR, EIMS) were in close agreement with that for (−)-kauran-16α-ol (**1**) [6–8] previously isolated from the fruit of *X. aethiopica* [3] and from *Annona senegalensis* Pers. [9].

Diterpene B (yield 0.034%) analysed for $C_{22}H_{32}O_4$, mp 217–222°, $[\alpha]_D^{25}$ −65°. The presence of an acetyl substituent was indicated by IR (1732 cm^{-1}), a fragment at *m/z* 300 $[M-OAc]^+$ and a signal at δ 2.05 (3H). Hydrolysis of B gave a compound identical to 7β-hydroxy-(−)-kaur-16-en-19-oic acid (**2**) isolated from *X. aethiopica* stem bark [4]. Diterpene B must therefore be 7β-acetoxy-(−)-kaur-16-en-19-oic acid (**3**) which does not appear to have previously been recorded as a natural product but has been synthesized [10].

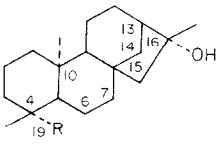
Diterpene C (yield 0.04%), obtained in an impure state, analysed for $C_{20}H_{28}O_3$ and gave a methyl ester. Both C and the ester had IR and ^{1}H NMR characteristics of a kaur-16-en-19-oic acid with an additional band at 1725 cm^{-1} in the IR indicating the third oxygen was present as a carbonyl. The non-equivalence of the vinylic protons of the exocyclic double-bond (δ 5.24, 5.95) suggested placement of the carbonyl at C-15. Further signals at δ 2.42 (H-14$_{eq}$) and 3.15 (H-13$_{eq}$) were also deshielded by the carbonyl and together with the major ion at *m/z* 148 $[C_{10}H_{12}O]^+$, formed by fission between C-9/C-10 and C-6/C-7, confirm placement of the carbonyl at C-15. On the basis of the above C can be characterized as 15-oxo-(−)-kaur-16-en-19-oic acid (**4**), previously recorded from *X. aethiopica* [3]. The lower mp, 168–173°, and $[\alpha]_D^{25}$, −160°, than previously recorded [3] may be attributed to the impure nature of the isolate.

Diterpene D (trace amounts) analysed for $C_{20}H_{32}O_3$, mp 270°, $[\alpha]_D^{25}$ −70°. The IR showed bands at 3450 and 1700 cm^{-1} for OH and COOH, and the ^{1}H NMR signals for three *tert.* methyl groups at δ0.96, 1.23 (for C-4 and C-10 methyls of a kauran-19-oic acid) and at 1.36 (for C-16 methyl). The strong deshielding of the latter suggested that the OH was also placed at C-16 and this was sustained by the absence of an oxymethine proton and by a major ion at *m/z* 262 $[C_{17}H_{26}O_2]^+$ for loss of ring-D. These data are in close agreement with those published [8] for 16α-hydroxy-(−)-kauran-19-oic acid (**5**), previously recorded as a fungal metabolite but not from higher plants.

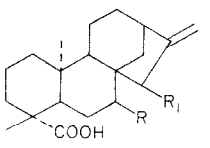
EXPERIMENTAL

IR spectra were run as KCl discs. ^{1}H NMR spectra were run at 90 MHz in $CDCl_3$ using TMS as int. standard. EIMS were obtained at elevated temp. at 70 eV. $[\alpha]_D$ were recorded in $CHCl_3$. Mps were uncorr. Petrol refers to the bp 40–60° fraction.

The powdered stem bark (50 g) was extracted with petrol.



1 R = Me
5 R = COOH



2 R = OH, R_1 = H_2
3 R = OAc, R_1 = H_2
4 R = H, R_1 = O

*Part 10 in the series "Chemical Studies in the Annonaceae". For Part 9 see ref. [11].

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The extract was concentrated and gave a ppt. of the dimer (360 mg). The supernatant was subjected to CC over Si gel and on elution with petrol–EtOAc (49:1) gave **1** (100 mg; found: M^+ 290. 2595; $C_{20}H_{34}O$ requires 290.2610) followed by more dimer (200 mg). Further elution with petrol–EtOAc (9:1) gave **3** (20 mg; found: M^+ 316.2029; $C_{20}H_{28}O_3$ requires 316.2038) followed by **4** (17 mg; found: M^+ 360.2326; $C_{22}H_{32}O_4$ requires 360.2300). Finally elution with petrol–EtOAc (4:1) gave a mixture which on prep. TLC (Si gel: solvent, toluene–EtOAc–HOAc 40:9:1) yielded **5** (R_f = 0.23, 5 mg; found: M^+ 320.2359; $C_{20}H_{30}O_0$ requires 320.2351).

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ERGOSTEROL, THE UNUSUAL DOMINANT STEROL OF THE PYTHIACEOUS FUNGUS *ZOOPHAGUS INSIDIANS*

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Key Word Index—*Zoophagus insidians*; Pythiaceae; fungi; ergosterol; sterols.

Abstract—The pythiaceous fungus, *Zoophagus insidians*, synthesizes ergosterol as its dominant sterol. This observation is unusual because the Pythiaceae were previously throught to be incapable of sterol synthesis and the pantonemic fungi in general to not produce ergosterol.

INTRODUCTION

Fungi may be described as either pantonemic or non-pantonemic. The former group consists of the Oomycetes and Hyphochytriomycetes, which are considered by most mycologists to have a phylogenetic ancestry different from all other fungi[1]. Members of these fungal classes usually possess cholesterol and the 24-alkylidene cholesterols as dominant sterols[2–6] although a few may possess 24-alkyl sterols[7]. In contrast, the non-pantonemic fungi generally possess ergosterol and its precursors as their major sterols[2–4, 8, 9], although some may possess sterols of the stigmastane[10, 11] or cholestane[7, 12] series. Some of the Oomycetes, such as *Lagenidium giganteum* and the pythiaceous fungi *Pythium* and *Phytophthora*, are unable to synthesize sterols and must depend upon an exogenous source to fulfill metabolic requirements[4, 6, 13].

The pantonemic fungi are considered by some to have arisen from pantonemic algal ancestors as they

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