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ACUTIFLORIC ACID: A DITERPENE DIMER FROM THE STEM BARK OF *XYLOPIA ACUTIFLORA**

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Key Word Index—*Xylopia acutiflora*; Annonaceae; diterpene dimer; acutifloric acid; kaurane and labdane precursors

Abstract—In addition to four kaurane and kaurene diterpenes, the stem bark of *Xylopia acutiflora* yielded a dimeric diterpene derived via Diels–Alder condensation of kaurene and labdane monomers. The structure of the dimer, which has been given the trivial name acutifloric acid, was assigned on the basis of detailed spectroscopic analysis.

INTRODUCTION

Xylopia acutiflora (Dunal) A. Rich. is a shrub or small tree found in the lowland forests of west Africa [2]. In a previous paper [3] we reported the isolation of four diterpenes from a sample of stem bark collected in the Korup National Park, Cameroun; namely (−)-kauran-16-ol, 7β-acetoxy-(−)-kaur-16-en-19-oic acid, 15-oxo-(−)-kaur-16-en-19-oic acid and 16α-hydroxy-(−)-kauran-19-oic acid. During the extraction of the above, a fifth compound was obtained. In this paper we report on the identification of this compound as a novel diterpene dimer to which we have assigned the trivial name acutifloric acid.

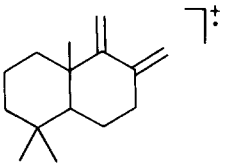
RESULTS AND DISCUSSION

Acutifloric acid separated from the concentrated petrol extract in a yield of 1.1% and was recrystallized from petrol as fine needles, mp 222–227°, $[\alpha]_D$ −127° (*c* 1.0; $CHCl_3$). Accurate mass measurement indicated the empirical formula $C_{40}H_{60}O_3$ (Found: $[M]^+$ at *m/z* 588.4517; required: 588.4542) and the IR spectrum (KCl disc) showed bands for an exocyclic double bond (3080, 1640 cm^{-1}), a carbonyl (1730 cm^{-1}) and a carboxylic acid (1700 cm^{-1}). The presence of the carbonyl was confirmed by reduction to the corresponding alcohol with lithium aluminium hydride (gum: Found: $[M]^+$ at *m/z* 590.4698; $C_{40}H_{62}O_3$ requires: 590.4699; IR ν_{max} cm^{-1}: 3400, 3070, 1700) and that of the carboxylic acid by formation of the methyl ester using ethereal diazomethane (mp 137–140°, Found: $[M]^+$ at *m/z* 602.4732; $C_{41}H_{62}O_3$ requires: 602.4699).

The ^1H NMR spectrum (360 MHz, $CDCl_3$) showed the presence of five methyl groups as singlets at δ0.68, 0.80, 0.87, 1.02 and 1.26, confirmed the exocyclic double bond by two singlets (1H each) resonating at 4.50 and 4.82, and revealed an olefinic proton as a singlet at 5.26. The ^{13}C NMR spectrum (90.56 MHz, $CDCl_3$) showed six downfield resonances: at δ106.1 (*t*) and 148.5 (*s*) for the exocyclic double bond, at 117.5 (*d*) and 138.0 (*s*) for a trisubstituted double bond, and at 183.8 and 227.1 (both *s*) for carboxylic acid and carbonyl, respectively. The above data showed acutifloric acid to have four centres of unsaturation, thus requiring a seven-ring structure for the dimer.

The ^1H NMR resonances for methyl groups at δ0.68, 0.80 and 0.87 and those for the exocyclic double bond were in close accord with published data for diterpenes with a labda-8-en skeleton [4, 5], the highly shielded 0.68 resonance being attributable to an α (axial) C-10 methyl. The presence of this system was also indicated by the electron impact mass spectrum, which gave a base peak at *m/z* 204 $[C_{15}H_{24}]^+$, which can be assigned to the bicyclic

*Part 13 in the Series "Chemical Studies in the Annonaceae". For Part 12 see ref. [1].



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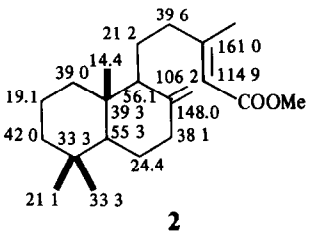

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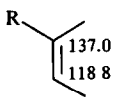

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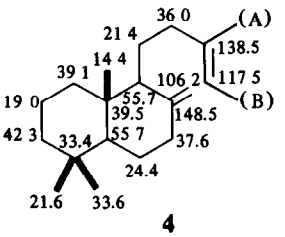

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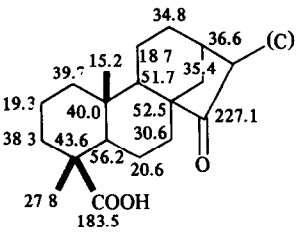

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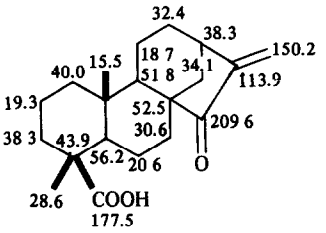


6

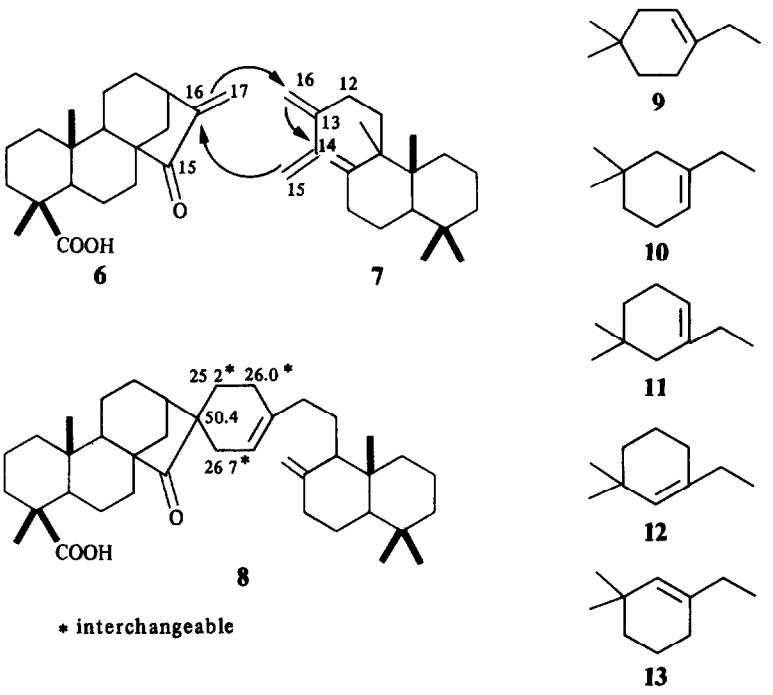
nucleus (**1**) of a non-oxygenated labda-8-en skeleton [6]. A comparison of ^{13}C NMR resonances reported [7] for methyl labda-8,13-diene-15-oate (**2**) with signals for acutifloric acid (see partial structure **4**) showed a very close similarity for all parts of the labdane skeleton other than those where the 15-carboxymethyl substituent of **2** would influence resonance values. In those positions, close accord was seen between acutifloric acid resonances and those reported [8] for the 1,2-dimethylethylene unit (**3**) which forms part of some labdanes. On the basis of these arguments acutifloric acid was assigned the partial structure **4**, that is, a non-oxygenated labdane that must be linked through positions A and B on **4** to a second diterpene unit which contains both CO and COOH groups.

The second diterpene unit showed spectral characteristics typical of the kaur-19-oic acids (axial COOH) previously reported from this species [3]. Thus, on formation of the methyl ester the ^{1}H NMR resonances for the two remaining methyl groups were shielded, from δ1.02 and 1.27 to 0.99 and 1.18 [9], and the IR spectrum showed a strong band at 1160 cm^{-1} [10]. Both the very strongly deshielded resonance in the ^{13}C NMR (δ227.1) and the IR band at 1730 cm^{-1} suggested that the carbonyl formed part of a cyclopentanone system [11]. These data were in agreement with the presence of a tetracyclic diterpene of the kaurane type having structure **5** with linkage to the remainder of the dimer through C. Further evidence in favour of this comes from the previous isolation of **6** from this bark sample [3] and from the good agreement between the ^{13}C NMR resonances reported for **6** [12] and signals for the dimer, other than those adjacent to the C-16 exocyclic double bond in **6**.

Thus, from the spectral evidence discussed above, acutifloric acid would appear to consist of a labdane (**4**) linked to a 15-oxo-kauran-19-oic acid (**5**) through the three positions A, B of **4** and C of **5**. These units together account for six rings and the linkage between the two must result in a seventh. Remaining ^{13}C NMR signals unaccounted for were those of the olefinic double bond, triplets at δ25.2, 26.0 and 26.7, and a singlet at 50.4. Of these, the 50.4 singlet must be placed at C-16 of the kaurane, where it will be deshielded by the adjacent carbonyl. That the C-16 carbon is quaternary was confirmed by the ^{1}H NMR spectrum of the alcohol produced on reduction of acutifloric acid which showed the resulting oxymethine proton to be a sharp singlet.

From the above arguments the seventh ring can only be rationalized as a cyclohexene ring. From a biogenetic viewpoint, the generation of the cyclohexene system could proceed through a Diels–Alder condensation between **6**, known to be present in this plant material, and the labdane derivative sclarene (**7**) (Scheme 1). In theory it is possible to envisage six structures (**8**–**13**) that could result from a condensation between these two monomers. Of the six potential structures for acutifloric acid, **8** is mechanistically most likely and **12** and **13** can be discounted because the broad signal for the olefinic proton in the ^{1}H NMR spectrum indicates coupling to an adjacent proton or protons.

Unfortunately it has so far proved impossible to resolve the position of the double bond in the cyclohexene ring



Scheme 1.

without ambiguity. Whilst acutifloric acid crystallized readily from a range of solvents, the crystals obtained to date have proved to be unsatisfactory for X-ray analysis.

Dimeric diterpenes appear to be rare in nature. Maytenone, from *Maytenus dispermus* [13], is almost certainly a product derived by Diels–Alder condensation in a manner comparable to acutifloric acid. On the other hand, the recently reported [6] acritopappus-lactones A and B, from *Acritopappus morii*, link two monomers through an ether bridge.

EXPERIMENTAL

Plant material. A voucher specimen, D. W. Thomas 595, has been deposited at the Herbarium, Royal Botanic Gardens, Kew.

Extraction of acutifloric acid. The powdered stem bark (50 g) was extracted with petrol (bp 40–60°). The extract was concd and on standing gave acutifloric acid (360 mg), crystallized from petrol (bp 60–80°). CC of the supernatant over silica gel gave, on elution with petrol–EtOAc (49:1), kauran-16α-ol [3] followed by further acutifloric acid (200 mg).

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