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ISOFRAXINELLONE, A LIMONOID LACTONE FROM THE BARK OF *FAGAROPSIS GLABRA*

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Abstract—Two lactones, both degraded limonoids, have been isolated from the trunk bark of *Fagaropsis glabra*. Isofraxinellone is a new natural compound and fraxinellone has previously been characterized in plants of the same family. The structure of isofraxinellone was determined by physical and chemical methods.

INTRODUCTION

Terpenoids resulting from the biochemical degradation of limonoids, are sometimes encountered in the Rutaceae [1–3]. During the study of a petrol extract of the trunk bark of *Fagaropsis glabra*, we isolated two degraded limonoid lactone derivatives. These were isofraxinellone (**1**), a new compound, and its isomer, fraxinellone (**2**), the latter previously found in plants of the same family [4–6].

RESULTS AND DISCUSSION

Isofraxinellone (**1**), the major component of the extract, was isolated as a slightly yellowish oil. The mass spectrum showed a very different fragmentation, compared to fraxinellone (**2**) [5]. The different structures of compounds **1** and **2** were confirmed by their IR spectra; isofraxinellone did not show the 1675 cm^{-1} band corresponding to conjugation between a carbonyl group and an intracyclic double bond.

Comparison of the ^{1}H NMR spectrum of **1** with that of synthetic or natural compounds of the limonoid type [5–11], confirmed the C-6 position of the double bond.

We have examined the relative configuration of assymetric carbons and of their substituents. Long distance couplings were evidenced by the allylic couplings (H-6, H-7a and H-6, Me-7) and homoallylic couplings (H-5, H-7a and H-5, Me-7). The allylic coupling constant ($J = 2$ Hz) suggested, for the dihedral angle of the A- and B-rings, a value consistent with an angle of between 40° and 60°. This did not permit a decision to be made between a *cis*- or *trans*-configuration of the ring system.

The ORD spectrum showed a characteristic positive Cotton effect [12] and, from previous work [13–15] dealing with the absolute configuration of lactones and their corresponding Cotton effects, we deduced the presence of an A/B *cis*-junction in **1**. This was confirmed by the ^{1}H NMR spectrum. The chemical shift of the Me-3a protons for isofraxinellone [d0.86 (*s*)] was similar to the value for the corresponding A/B *cis*-rings of the synthetic compound [δ0.84 (*s*)] [11].

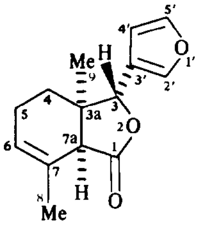
Moreover, the ^{1}H NMR spectrum of the compound resulting from the Diels–Alder reaction of maleic anhydride with the furan ring of **1**, did not show any signals due to the endo-isomer [16]. However, interpretation of the signals for the H-3, H-7a, H-2′, H-5′ and Me-3a protons showed the existence of two exo-adducts (*ca* 1:2 mixture), resulting from an attack from above or below the furan ring to give **3** or **4**, respectively. The signals of the spatially nearest protons (i.e. H-3, H-7a, H-2′, H-5′ and Me-3a) were shifted and provided more proof for the *cis*-junction. If the ring juncture had been *trans*, H-7a would be spatially too far away to be sensitive to the influence of one or other of these adducted parts. Thus, the relative configuration of the angular C-7a was proved.

As mentioned in a previous paper [11] on the synthetic compounds with an A/B *cis*-ring juncture, when the Me-3a substituent and the furan ring are in a relative *cis*-position the value of the Me-3a signal is δ0.84. When both substituents are in a relative *trans*-position the chemical shift for the Me-3a signal is δ1.08 [11]. In the natural product **1**, the value of δ0.86 (*s*) suggested a relative *cis*-configuration for the Me-3a and the furan ring.

Isomerization of isofraxinellone with sodium hydroxide gave a compound identical in all respects (^{1}H NMR, ORD), with a fraxinellone [10, 11] sample isolated from a trunk bark extract. This result showed that the relative configurations of C-3 and C-3a were the same in **1** and **2**.

The easy conversion of isofraxinellone into its isomer fraxinellone raised questions about the natural origin of one or other of the products. For this purpose, we studied the ^{1}H NMR spectrum of the crude extract. The spectrum clearly showed that isofraxinellone was the major component in the extract, however, fraxinellone was also present (relative abundance 85:15, respectively). This relative abundance did not change with extraction time, heating nor after prolonged contact with silica gel.

The natural origin of fraxinellone and isofraxinellone was, thus, established and isofraxinellone appears to be a biosynthetic intermediate. The stereochemical correlation between limonin and its derivative, fraxinellone, has been shown [2, 3, 17]. Cassady and Liu [18] isolated calodendrolide (**6**) and, based on its structure, inserted it as a biosynthetic intermediate in the transformation limonin → fraxinellone. They proposed the transformation of calodendrolide (**6**) into fraxinellone through isofraxinellone. As a result, the isolation and characterization of



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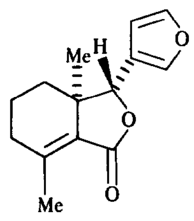

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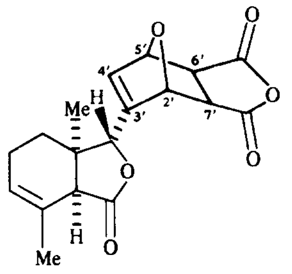

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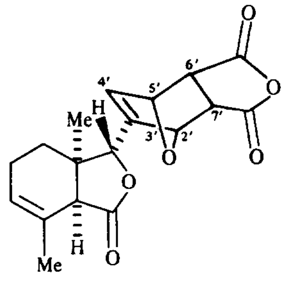

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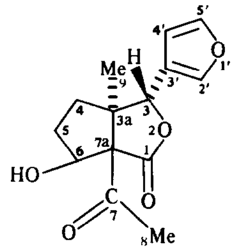

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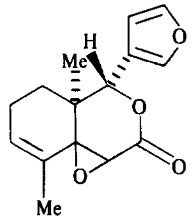

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isofraxinellone is of some importance in explaining this biosynthetic pathway.

EXPERIMENTAL

Plant material. Fagaropsis glabra Capuron trunk bark was collected in the Sambava country (NE. coast of Madagascar) and authenticated at source by the ORSTOM centre of Tananarive.

Extraction and isolation. Powdered trunk bark (2 kg), extracted with petrol bp 40–60° (5 l.) for 2 days in a stainless steel percolator gave 20.3 g crude extract. An aliquot (9.7 g) was chromatographed over silica gel (Merck). Isofraxinellone was eluted with petrol–C_6H_6 (4:1) and fraxinellone was then eluted with petrol–C_6H_6 (1:1).

3α-(3′-*Furanyl*)-3aα,7-*dimethyl*-3a,4,5,7a-*tetrahydroisobenzofuranone* (**1**). Oil. (Found: C, 73.78; H, 6.33. $C_{14}H_{16}O_3$; requires: C, 72.41; H, 6.89 %.) $[\alpha]_D^{20}$ + 19.6° ($CHCl_3$; *c* 17.0); IR ν_{max}^{KBr} cm^{-1}: 1760 (C=O), 3020 (C=C), 3140, 1600, 1500, 1025, 870, 800 (furan); ^{1}H NMR TMS (100 MHz, $CDCl_3$): δ0.86 (3H, *s*, Me-3a), 1.62 (2H, *m*, H-4), 1.93 (3H, *br s*, Me-7), 2.13 (2H, *m*, H-5), 2.73 (1H, *br s*, H-7a), 5.18 (1H, *s*, H-3), 5.62 (1H, *m*, H-6), 6.33 (1H, *s*, H-4′), 7.42 (2H, *s*, H-2′, H-5′); EIMS 70 eV, *m/z* (rel. int.): 232 $[M]^+$ (100), 188 $[M-CO_2]^+$ (21), 187 $[M-CO_2H]^+$ (19), 173 $[M-CO_2-Me]^+$ (38), 159 $[M-CO_2H-CO]^+$ (15), 145 $[M-CO_2-Me-CO]^+$ (20), 108 $[M-C_4OH_3-C_2O_2H]^+$ (47), 93 $[M-C_4OH_3-C_2O_2H-Me]^+$ (62); ^{13}C NMR (20.115 MHz, $CDCl_3$): δ175.3 (C-1), 143.4 (C-5′), 139.7 (C-2′), 127.1 (C-7), 123.4 (C-6), 121.1 (C-3′), 108.7 (C-4′), 79.9 (C-3), 51.0 (C-7a), 41.6 (C-3a), 28.8 (C-4), 21.9, 21.7, 20.8 (C-5, C-8, C-9 undetermined).

3α-(3′-*Furanyl*)-3aα,7-*dimethyl*-3a,4,5,6-*tetrahydroisobenzofuranone* (**2**). Mp 110–112°. (Found: C, 71.89; H, 7.01.) $[\alpha]_D^{20}$ −40.6° ($CHCl_3$; *c* 11.9); IR ν_{max}^{KBr} cm^{-1}: 1740 (C=O), 1675 (conjugated C=C–C=O); ^{1}H NMR and EIMS identical with the lit. [5]; ^{13}C NMR (20.115 MHz, $CDCl_3$): δ169.5 (C-1), 148.2 (C-7a), 143.2 (C-5′), 139.5 (C-2′), 127.2 (C-7), 120.5 (C-3′), 108.4 (C-4′), 83.2 (C-3), 41.6 (C-3a), 32.0 (C-4), 31.6 (C-6), 20.3, 18.4, 18.2 (C-5, C-8, C-9 undetermined).

Double-bond isomerization. Compound **1** (200 mg) was dissolved in EtOH (5 ml) and 2 M NaOH (5 ml). After steam distillation, the collected aq. phase was extracted with Et_2O

(3 × 5 ml), then acidified with HCl and again extracted with Et_2O (3 × 5 ml). Fraxinellone (163 mg) was obtained as crystals: mp 108–109°; $[\alpha]_D^{20}$ $-40.3°$ ($CHCl_3$; *c* 12.4), ^1H NMR identical with that of the natural sample.

Epoxidation of isofraxinellone. Epoxidation of **1** (198 mg) in $CHCl_3$ (50 ml) was achieved with *p*-nitroperbenzoic acid (200 mg) for 12 hr. The ppt was removed by filtration and the organic phase washed with 10% NaOH (3 × 50 ml), then with H_2O (3 × 50 ml) and dried on Na_2SO_4. The β-epoxide (136 mg), was collected as white crystals: mp 112–114°; IR ν_{max}^{KBr} cm^{-1}: 1760 (C=O); ^1H NMR (60 MHz, $CDCl_3$); δ0.86 (3H, *s*, Me-3a), 1.67 (2H, *m*, H-4), 1.55 (3H, *s*, Me-7), 1.93 (2H, *m*, H-5), 2.60 (1H, *s*, H-7a), 3.08 (1H, *t*, $J_{H\text{-}5\text{-}H\text{-}6}$ = 2 Hz, H-6), 5.07 (1H, *s*, H-3), 6.27 (1H, *s*, H-4'), 7.43 (2H, *s*, H-2', H-5'); MS *m/z*: 248 $[M]^+$ 233, 232, 230, 125, 107, 96.

Oxidative breaking of the $\Delta^{6(7)}$*-double bond.* Compound **1** (290 mg) was dissolved in *t*-BuOH (200 ml) and 0.36 M Na_2CO_3 (30 ml), 0.1 M $KMnO_4$ (200 ml) and 0.25 M $NaIO_4$ (40 ml) was added. The mixture was stirred for 2 hr at room temp. and then extracted with C_6H_6 (3 × 150 ml). The organic phase was washed with satd $NaHCO_3$ (75 ml), then with H_2O. Drying on Na_2SO_4 and concn gave 103 mg ketol **5**: IR ν_{max}^{KBr} cm^{-1}: 1760 (C=O lactone), 1700 (C=O ketone), 3450 (OH); ^1H NMR (60 MHz, $CDCl_3$): δ0.89 (3H, *s*, Me-3a), 1.66–2.20 (4H, *m*, H-4, H-5), 2.3 (3H, *s*, COMe-7), 3.42 (1H, *s*, D_2O exchange, OH-6), 4.83 (1H, *t*, H-6), 5.43 (1H, *s*, H-3), 6.37 (1H, *s*, H-4'), 7.47 (2H, *s*, H-2', H-5').

Acetylation of the secondary alcohol of **5**. The ketol derivative was acetylated with Ac_2O–pyridine (2:1) to give 53 mg **5** as an oil: IR ν_{max}^{KBr} cm^{-1}: 1760 (C=O lactone), 1740 (C=O ester), 1700 (C=O ketone); ^1H NMR (60 MHz, $CDCl_3$): δ0.90 (3H, *s*, Me-3a) 1.7–2.0 (4H, *m*, H-4, H-5), 2.07 (3H, *s*, $MeCO_2$), 2.40 (3H, *s*, Me-7), 5.37 (1H, *s*, H-3), 5.80 (1H, *t*, H-6), 6.38 (1H, *s*, H-4'), 7.53 (2H, *s*, H-2', H-5'); MS *m/z*: 306 $[M]^+$, 263, 246, 223, 203, 183, 139.

Bromination of isofraxinellone. Bromination of **1** (125 mg) in refluxing CCl_4 (30 ml) was performed in the presence of a catalytic amount of benzoyl peroxide and 125 mg freshly recrystallized *N*-bromosuccinimide. The mixture was irradiated with a UV lamp (10 min). After cooling, petrol was added (30 ml) and the filtered soln was washed with 10% $NaHCO_3$, H_2O and then dried on $MgSO_4$. Chromatography over silica gel with C_6H_6 as eluent gave 30 mg the C-5 position monobrominated homoannular diene: IR ν_{max}^{KBr} cm^{-1}: 1760 (C=O), 1640 (C=C homoannular diene); UV λ_{max}^{EtOH} nm: 271; ^1H NMR (60 MHz, $CDCl_3$): δ1.0 (3H, *s*, Me-3a), 2.3 (3H, *s*, Me-7), 3.1 (1H, *s*, H-7a), 4.9 (1H, *s*, H-3), 5.83 (1H, *s*, H-4), 6.03 (1H, *s*, H-6), 6.4 (1H, *s*, H-4'), 7.5 (2H, *s*, H-2', H-5'); MS *m/z*: 309, 307 $[M-H]^+$, 213, 211, 186, 184, 122, 105, 97.

Condensation of maleic anhydride with **1**. Maleic anhydride (600 mg) and **1** (450 mg) were dissolved in C_6H_6 (5 ml). The soln was heated (15 min) under reflux and left to stand at room temp. for 2 days. Recrystallization in petrol–C_6H_6 (50:50) gave 277 mg white crystals: mp: decomposes; $[\alpha]_D^{20}$ $-7.9°$ ($CHCl_3$; *c* 12.4); IR ν_{max}^{KBr} cm^{-1}: 1760 (C=O lactone), 1785, 1865 (C=O anhydride); ^1H NMR (60 MHz, $CDCl_3$): δ1.05, 1.10 (3H, overlapping *s*, *s*, 33% Me-3a, 67% Me-3a) 1.60 (2H, *m*, H-4), 1.86 (3H, *br s*, Me-7) 2.10 (2H, *m*, H-5), 2.63, 2.80 (1H, *br s*, *br s*, 33% H-7a, 67% H-7a), 3.40 (2H, *s*, H-6', H-7'), 4.80, 5.43 (2H, *d*, *d*, *J* = 2, 2 Hz, 33% H-2', 33% H-5', 67% H-2', and 67% H-5'), 5.00, 5.33 (1H, *s*, *s*, 33% H-3, 67% H-3), 5.63 (1H, *m*, H-6), 6.40 (1H, *m*, H-4').

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