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4-EPIISOCOMMUNIC ACID AND AMENTOFLAVONE FROM *CALLITRIS RHOMBOIDEA*

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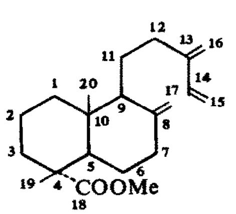
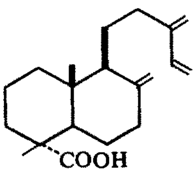
Key Word Index—*Callitris rhomboidea*; Cupressaceae; leaves; 4-epiisocommunic acid; amentoflavone.

The leaves of *Callitris rhomboidea* were previously examined for their essential oil content [1]. The present report deals with the isolation and characterization of a biflavonoid and a new diterpene acid. The residue from the combined petrol and benzene extracts showed one major acidic compound (TLC and colour tests) and the acid was partially extractable into aqueous sodium hydroxide. However, the isolation of the free acid was found to be difficult. Hence it was isolated as its methyl ester. Its MW, 316 (MS), is consistent with a molecular formula $C_{21}H_{32}O_2$ of a diterpene. The UV absorption spectrum of the compound showed the presence of a monosubstituted butadiene chromophore (224 nm). It readily formed an adduct with maleic anhydride. Its IR spectrum showed a terminal vinyl (990 cm^{-1}), an exocyclic methylene (890 cm^{-1}) and conjugation (1648 and 1595 cm^{-1}). The NMR spectrum indicated two tertiary methyls, six olefinic protons and a multiplet centered at δ 6.2, characteristic of the X-proton constituting an ABX system. In its MS, intense peaks at *m/e* 181 and 121 were observed. They were indicative of the bicyclic nature of the diterpene having an exocyclic methylene at C-8 [2]. A C_6H_9 side chain at C-9 was also indicated by the fragment at *m/e* 235 ($M^+ - 81$). With the help of these data, the structure of the methyl ester was formulated as 1.



1

The equatorial nature of the ester group, indicated by a strong band at 1245 cm^{-1} in the IR spectrum [3], was confirmed by its easy saponification to the acid (2). The hexahydro compound obtained readily by hydrogenation of 1 over palladium, was reduced with LAH and the alcohol (3) showed the NMR signals expected for an equatorial —$C\underline{H}_2OH$ [4]. The alcohol was converted through its tosylate into the hydrocarbon (4), identical



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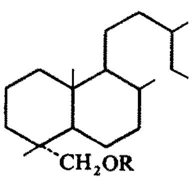
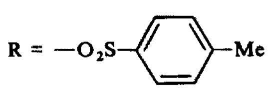
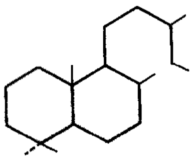


3a. R = H

3b.





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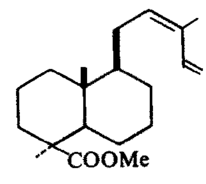

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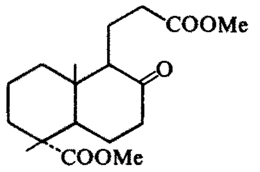
with the hydrocarbon similarly prepared from methyl communate (5). The position of double bonds in the compound was proved by OsO_4–$NaIO_4$ oxidation of 1. In addition to formaldehyde an acid was obtained. The spectral properties of its methyl ester (NMR and MS) were consistent with the structure 6. The above observations support structure 2 for the diterpene acid.

The alcoholic extract gave on chromatography over Si gel, a pale yellow compound. Its solubility properties, TLC mobility, colour tests and UV spectrum suggested it to be a biflavone. It was identified as amentoflavone based on the analysis of the NMR spectra of its acetate and methyl ether and finally confirmed by comparison with an authentic sample of amentoflavone, its hexacetate and hexamethyl ether.



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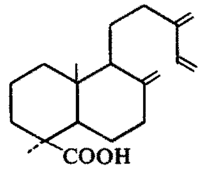

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Sawada [5] had identified hinokiflavone (C—O—C linked biflavone) in *C. glauca* which was the only species examined for biflavones out of 15 species. *C. rhomboidea* is the second species now examined, showed amentoflavone (C—C linked biflavone) and no other biflavone was detected. *C. glauca* (= *C. columellaris*) was known to contain isocommunic acid (7) [6] and so the name 4-epiisocommunic acid is appropriate for the new diterpene acid. Isocommunic acid was not detected in *C. rhomboidea*.

EXPERIMENTAL

The purity of samples, where needed, was established by Si gel TLC and $AgNO_3$–Si gel TLC.

Methyl-4-epiisocommunate. The air dried leaves of *Callitris rhomboidea* (1 kg: obtained from National Botanical Gardens, Ooty, India) were extracted successively with petrol, C_6H_6 and EtOH (3 × 3 l. 10 hr each). The petrol and C_6H_6 extracts were pooled and conc under red. press. The green syrupy residue was methylated (CH_2N_2) and subjected to chromatography over a column of Si gel–$AgNO_3$ (25 %). Elution with petrol–C_6H_6 (9:1), yielded methyl-4-epiisocommunate (1) as a pale yellow liquid (1 g). $R_f = 0.75$ (C_6H_6); $[\alpha]_D^{25} = +45°$ (*c*, 0.5 in $CHCl_3$); UV λ_{max}^{MeOH} nm: 224 (log ε, 4.3); IR (film) cm^{-1}: 1730, 1648, 1595, 1310, 1245, 990 and 890. NMR (CCl_4): δ 0.6(3H, *s*, C-10 Me), 1.1(3H, *s*, C-4 Me), 4.5–5.3 (6H, *m*, C-8, C-13 and C-14 methylenes) and 6.2(1H, *m*, C-14). MS *m/e* (rel. int.): 316 (M^+, 5.2 %), 257 (5.8), 249 (6.0), 241 (15.8), 235 (20.4), 189 (16.5), 181 (18.4), 175 (12.7) and 121 (100). The maleic anhydride adduct was obtained as a liquid. IR (film) cm^{-1}: 2985, 1786, 1724, 1460, 1429, 1250, 935 (broad) and 893; NMR(CCl_4): δ 0.58(3H, *s*, C-10 Me), 1.2(3H, *s*, C-4 Me), 3.4(2H, broad *s*, adduct methines), 3.65(3H, *s*, C-4 COOMe), 4.5–4.9 (1H, each, 2 *br s*, C-17) and 5.75 (1H, broad signal, $W_{1/2} = 10$ Hz, [6] C-14).

4-Epiisocommunic acid. Hydrolysis of methyl-4-epiisocommunate by MeOH–KOH (10 %, 3 hr), yielded 4-epiisocommunic acid (2) as a white solid, mp 98–100°; $[\alpha]_D^{25} = +42°$ (*c*, 0.65 in $CHCl_3$); IR(KBr) cm^{-1}: 2959, 1695, 1645, 1595, 1460, 1379, 1258, 990 and 895.

OsO_4–$NaIO_4$ oxidation. Oxidation of (1) by OsO_4–$NaIO_4$ was carried out according to the standard procedure. The product was stream distilled and formaldehyde was identified in the distillate (chromotropic acid and dimedone derivative). The residue was extracted with Et_2O and the Et_2O soluble portion was methylated (CH_2N_2). The methyl ester 6 (liquid) was purified by PTLC using C_6H_6, $R_f = 0.22$. IR(film) cm^{-1}; 2941, 1724 (broad), 1493, 1439, 1370, 1245, 1163, 1105 and 1042. NMR (CCl_4): δ 0.65(3H, *s*, C-10 Me), 1.15(3H, *s*, C-4 Me), 2.31(5H, *m*) and 3.7 and 3.75 (6H, 2*s*, 2 × −COOMe). MS *m/e* (rel. int.): 309 ($M^+ - 15$, 32 %), 277 (35.5), 217 (27), 181 (5.2) and 121 (100).

Conversion of methyl 4-epiisocommunate to (4). Methyl 4-epiisocommunate (1, 0.2 g) in dry EtOAc (10 ml) was hydrogenated over Pd–C (10 %). Hexahydro compound was obtained as a liquid (0.2 g), $[\alpha]_D^{25} = +39.6°$ (*c*, 0.5 in $CHCl_3$): IR(film) cm^{-1}: 1709, 1439, 1242 and 962. The hexahydro compound (0.2 g) in dry THF (20 ml) was treated with LAH (0.2 g). After refluxing for 4 hr excess LAH was destroyed by dil. H_2SO_4 and the product was extracted into Et_2O. Removal of Et_2O furnished a gummy liquid (3a) (0.12 g); IR(film) cm^{-1}: 2945, 1481, 1449, 1370 and 1040. NMR(CCl_4): δ 3.25(2H, *dd*, $J = 11$ Hz, C-4 CH_2OH, equatorial). The alcohol (3a) (0.1 g) was tosylated using C_5H_5N (3 ml) and *p*-toluene sulphonylchloride (0.1 g). Usual work up provided the tosylate (3b) as a pale yellow liquid (0.1 g); NMR (CCl_4): δ 2.3(3H, *s*, Ar–Me), 3.6(2H, *m*, —$C\underline{H}_2OTs$) and 7.4 and 7.8(2H each, 2 *d*, $J = 9$ Hz, aromatic protons of the tosyl group). The tosylate (3a 0.1 g) was refluxed with LAH (0.1 g) in dry dioxan (10 ml) for 3 hr. Work up provided a waxy solid (4, 0.02 g) purified by PTLC on Si gel using petrol–C_6H_6 (1:1). $[\alpha]_D^{25} = +22°$ (*c*, 0.52 in $CHCl_3$). IR (film) cm^{-1}: 1470, 1390, 1200, 980 (broad), 820 and 780. The hydrocarbon was found to be identical (co TLC, superimposable IR and specific rotation) with the one prepared from methylcommunate (5).

Conversion of methyl communate (5) *to* (4). The above sequences of reactions were also carried out on methyl communate (5). Hydrogenation over Adam's catalyst yielded the hexahydromethyl communate as a liquid. It was reduced with LAH in THF, to obtain the alcohol (4) as a glassy solid. NMR(CCl_4): δ 3.6 (2H, *dd*, $J = 11$ Hz, C-4 CH_2OH axial). The tosylate ester was reacted with LAH in dry dioxan. The hydrocarbon obtained was purified by PTLC using petrol–C_6H_6 (1:1).

Isolation and identification of amentoflavone. The alcoholic extract was conc under red. press. and the greenish residue so obtained was macerated with boiling petrol and C_6H_6 (total 1 l. each) to remove chlorophyll and other waxes. The remaining brownish mass gave colour tests typical for flavonoids (Mg–HCl; NH_3) and gave a dark brown colour with alcoholic $FeCl_3$. It was subjected to chromatography over a column of Si gel. Elution with $CHCl_3$–MeOH (24:1) afforded a pale yellow solid, crystallized from MeOH, shining crystals (0.5 g), mp > 350°; λ_{max}^{MeOH} nm: 272 and 334: IR (KBr) cm^{-1}: 3030, 1654. It formed an acetate (C_5H_5N Ac_2O, 110°, 6 hr) mp 245° and a methyl ether (dry methylation), mp 218–19°. The parent compound, its acetate and methyl ether were compared directly with amentoflavone, its hexaacetate and hexamethyl ether and found to be identical in all respects (mp, mmp, co-TLC and superimposable IR).

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STRUCTURE DE DEUX METHOXY-YOHIMBINES, ISOLEES DE *RAUWOLFIA CAPURONI*

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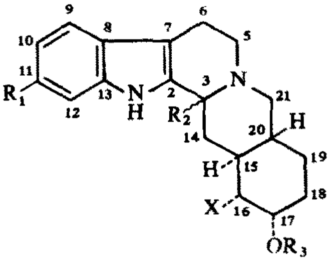
Key Word Index—*Rauwolfia capuroni*; Apocynaceae; structure; 11-methoxy-yohimbine; 11-methoxypseudoyohimbine; indole alkaloids.

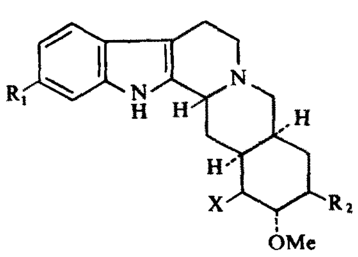
Par extraction des alcaloïdes des écorces de tronc d'une Apocynacée malgache *Rauwolfia capuroni* Mgf (C. Miet, M. Debray, M. Jacquemin et J. Poisson, Non publié) on obtient un totum basique soluble dans le chloroforme qui cristallise abondamment par concentration de la solution. Le produit obtenu **1** représente la presque totalité des alcaloïdes totaux (26 g. p. 1000)†. Toutefois, on peut isoler en très faible proportion des eaux-mères, par chromatographie sur alumine, un autre alcaloïde **2** qui cristallise dans le méthanol.

Après cristallisation répétée dans l'acétone, l'alcaloïde **1** se présente comme un produit homogène, F. 267–268° (Microscope Kofler), $[\alpha]_D^{20}$ + 30° (pyridine, c = 1), de formule $C_{22}H_{28}O_4N_2$. Sa solubilité est très faible dans les solvants usuels. Le spectre UV (EtOH) est du type méthoxy-6 indole **3**: λ_{max} nm (log ε) 229 (4,51), 271 (3,68), 298 (3,75) et le spectre IR (KBr) révèle la présence de groupes OH (3520 cm^{-1}), NH (3320 cm^{-1}) et carbonyle (1720 cm^{-1}). Le spectre RMN ^1H à 60 MHz (DMSO-d_6 + TMS) montre des signaux à δ ppm 3,68 (sing., 3 pr., $COOC\underline{H}_3$) et 3,76 (sing. 3 pr., Ar—$OC\underline{H}_3$) ainsi que 3 multiplets de 1 proton chacun dans la zône aromatique à 6,45 (doublet dédoublé, J_{HH} = 2 Hz et 8 Hz); 6,75 (doublet, J_{HH} = 2 Hz) et 7,10 (doublet, J_{HH} = 8 Hz) qui confirment le noyau méthoxy-6 indole **3**. On note également deux protons échangeables par D_2O à 4,41 (OH) et à 8,66 (N$\underline{H}$ indolique). Le spectre de masse [*m*/*e* (abondance %): 384 (100) (M^+), 383 (90), 369 (2), 354 (7), 353 (10), 214 (10), 200 (15), 199 (13), 186 (10), 174 (10), 173 (6)] possède le profil de celui de la yohimbine **4** avec un décalage de + 30 um des fragments contenant le noyau indole [1].

On peut donc envisager pour l'alcaloïde **1** la structure plane d'une méthoxy-11-yohimbine. Celle-ci, et en particulier la position du méthoxyle indolique, est confirmée par l'analyse du spectre de RMN ^{13}C dans le DMSO-d_6 effectuée parallèlement à celle de la yohimbine **4**: deux signaux de méthines aromatiques subissent un fort déplacement à champ fort et correspondent à C-10 (Δδ −14,26 ppm) et C-12 (Δδ −15,74 ppm). Un autre méthine aromatique est déplacé en sens inverse, le C-11 (Δδ + 36,92 ppm) et son degré de substitution



1	R_1: OMe	R_2: H(β)	R_3: H	X: CO_2Me
2	: OMe	H(α)	H	CO_2Me
4	H	H(α)	H	CO_2Me
5	H	H(β)	H	CO_2Me
6	OMe	H(β)	Ac	CO_2Me
9	OMe	H(α)	one-17	H
10	H	H(α)	one-17	H



7 R_1: H R_2: $C_6H_2(OMe)_3COO$
8 R_1: OMe R_2: $C_6H_2(OMe)_3COO$
X: CO_2Me

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† L'isolement et la description de cet alcaloïde ont fait l'objet du dépôt d'un pli cacheté à l'Académie de Pharmacie, Paris le 19.2.1973.