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A new ketosteroid from the bark of *Couropita guianensis* Aubl.

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Chemical examination of the bark of *Couropita guianensis* Aubl. furnishes a new ketosteroid, couropitone in addition to β-amyrin, βamyrone, β-amyrin acetate, stigmasterol, ergosta-4,6,8(14), 22-tetraen-3-one, β-sitosterol and its glyco-side.The structure of couropitone is established as stigmasta-4,23(*E*)-dien-3-one **1**.

Couropita guianensis Aubl. (Cannonball tree, Fam: Lecythidauae) is a native of South America and Trinidad and planted in India near temples for its beautiful flowers. The large size fruit requires more than a year to ripen and the seeds are embedded in a stinking pulp[1]. Various parts of this tree are reported to be used for the treatment of skin diseases[2,3]. Different parts of this tree have been examined earlier and reported to contain oils, phenolic substances, acids[4,5], coloured matter, anthocyanidin and flavonoid glycosides[6,7], terpenoids[8,9], alkaloids and others[10,11].

The bark of the tree available in the university campus was collecged, dried and powdered. The dry powder was repeatedly extracted with ethanol in a soxhlet apparatus. The concentrate from the combined ethanolic extract was fractionated into hexane, benzene and methanol successively. The residues obtained from the individual solvent fractions were chromatographed over silica gel columns. The eluate fractions on evaporation and crystallisation of the solids obtained therefrom gave in all eight compounds. One of these happened to be a new ketosteroid, couropitone whose structure has been established as stigmasta-4,23(*E*)-dien-3-one **1** by a study of its physical and spectroscopic data and chemical reactions. The other compounds were identified as β-amyrin, β-amyrone, β-amyrin acetate, stigmasterol, ergosta-4,6,8(14), 22-tetraen-3-one, β-sitosterol and its glycoside. Although β-amyrin was reported earlier from this species, the presence of β-amyrin acetate and β-amyrone have now been reported. Similarly the report on the presence of steroid conjugated ketones including the new derivative, couropitone is quite significant.

The new ketosteroid, named couropitone, was obtained from the hexane-benzene (3:7) column fractions of the hexane soluble material and crystallised from methanol as colourless needles, m.p. 103-04°; $[\alpha]_D$+38.18° (*c* 0.16 in $CHCl_3$). Its molecular formula was fixed as $C_{29}H_{46}O$ based on elemental analysis and molecular ion M^+ at m/z 410 in its EIMS. Its molecular formula and ^1H NMR spectrum suggested it to be a steroid derivative. The UV absorption (241 nm) indicated the presence of an α,β-unsaturated kketone moiety which was suported by its IR absorption at 1670 cm^{-1} and formation of 2,4-dinitrophenylhydrazone derivative as shining orange needles. The presence of single oxygen present in the molecule in the form of a ketone indicated 3-keto functionality in the place of ubiquitous 3β-hydroxyl. This also locates one double bond in its conjugation (Δ^1 or Δ^4) more likely at C-4 considering its UV maximum.

The fragment ions m/z 271 (M^+–side chain, 32%) and 269 (M^+–side chain–2H, 25%) suggested the presence of a ten-carbon side chain $C_{10}H_{19}$ with a double bond in it (cf. Chart 1). The ^1H NMR spectrum showed the presence of six methyls, two tertiary as singlets at δ 0.72 (18-H_3) and 1.18 (19-H_3), three secondary methyls at δ 1.05 (6H, d, *J*=7 Hz, 26-H_3 and 27-H_3), 0.84 (3H, d, *J*=7 Hz, 21-H_3) and a primary methyl at 0.89 (3H, t, *J*=7 Hz, 29-H_3) reminiscent of a stigmastane skeleton. The above methyl chemical shifts closely agree with those of 23(*E*)-stigmasta-5,23-dien-3β-ol but for the 19-H_3 which appeared at δ 1.16 as in stigmasta-4-en-3-one[14]. Two protons were noticed in the olefinic region, one at δ 5.70 as a singlet and the other at

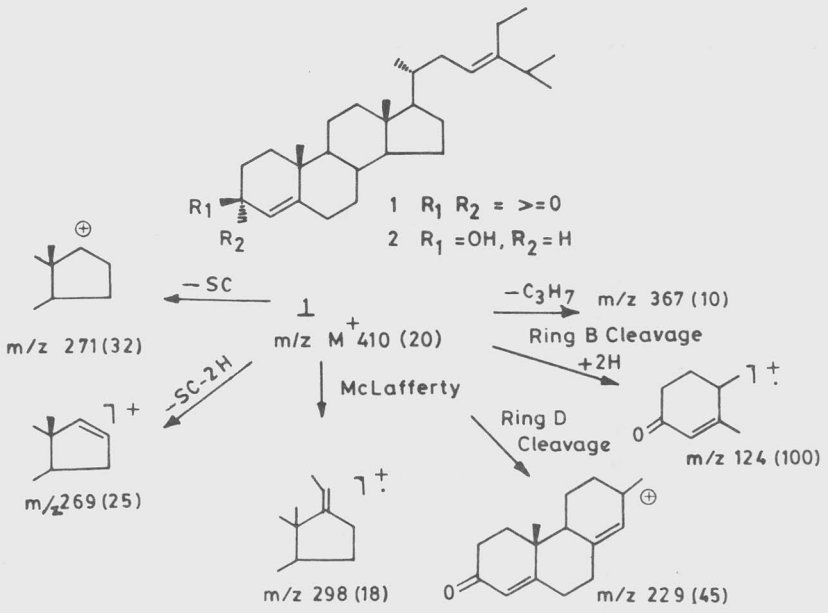

Chart 1

5.10 as a multiplet. The former could be assigned to the α-proton of an α,β-unsaturated ketone accounting for C_4-H. The second proton obviously corresponds to a trisubstituted double bond in the side chain. The absence of a methyl on double bond rules out the location of this double bond at $\Delta^{20(22)}$ or $\Delta^{24(28)}$ leaving it to be located at Δ^{23} position. The presence of 23-24 double bond was also supported by the fragment ion m/z 298 ($M^+-C_8H_{16}$) (cf. Chart 1) formed by McLafferty rearrangement with the transfer of 17-H and cleavage of 20-22 bond[13-18].

The configuration *E* or *Z* of D^{23} bond can be determined by the ^1H and ^{13}C NMR[19,20] chemical shifts of the side chain protons and carbons as assigned in cyclosadol and others[21,22]. In case of *Z*-configuration the isopropyl methane proton (25-H) appears deshielded at ≈ δ 2.2 compared to the same in *E*-isomers at ≈ δ 2.8. In couropitone the 25-H appeared mixed up in the multiplet at δ 2.3 with 28 methylene protons but no signal was found below 2.3 in support of *E*-configuration of Δ^{23}. The ^{13}C NMR spectrum of couropitone could not be obtained for want of sufficient sample. The foregoing evidence, however, fully supported the structure of couropitone as stigmasta - 4,23-(*E*)-dien-3-one **1**.

This compound has not been isolated so far from a natural source. It was however, reported as an intermediate by a series of reactions from fucosterol which is stigmasta-5,24(28)-dien-3β-ol[23]. The 23(*Z*) isomeric dienone was also reported from sargasterol[24]. Unfortunately, except for the melting points of the two dienones (110-111.5° and 121-122.5° respectively) no other physical or spectral characteristics are available. The m.p. of couropitone (103-04°) was found to be nearer to the ketone obtained from fucosterol suggesting their possible identity.

In order to provide further chemical evidence, couropitone was reduced with $NaBH_4$ in dry methanol to give an alcohol (over 80%). The alcohol **2**, $C_{29}H_{48}O$, crystallised from chloroform as colourless needles, m.p. 132-33°; $[\alpha]_D$ +64.04° and showed hydroxylic (3500 cm^{-1}) but no carbonyl absorption in its IR spectrum. It was found to be transparent in UV above 210 nm. Its ^1H NMR spectrum showed two trisubstituted olefinic protons, one at δ 5.10 as a multiplet for C_{23}-H and another at 5.30 as a doublet accounting for C_4-H of Δ^4-ene. Further, the C_3-αH was observed as a broad multiplet ($W_{1/2}$ = 20 Hz) at δ 4.21 as noticed in 3β- and 3α-hydroxy-Δ^4-cholestenes[25]. If it were a Δ^5-ene the C_3-α or β-H would have appeared around δ 3.5. Even in the alcohol **2** the isopropyl proton (25-H) appeared unaltered at δ 2.30 in support of 23(*E*)-configuration. The structure of alcohol **2**, which is again new in literature, could thus be derived as stigmasta-4,23(*E*)-dien-3β-ol.

Experimental Section

General. All melting points were determined on a VEB Analytik Dresden HMK hot plate and are uncorrected. Acme's Si gel-G was used for column and TLC. IR spectra were measured on a Shimadzu IR-408 spectrophotometer and UV spectra on a Milton Roy UV-vis spectrophotometer. ^{1}H NMR spectra were recorded on a Perkin-Elmer R-32 and Jeol-Ex 90, 90 MHz NMR spectrometers in $CDCl_3$ with TMS as internal standard (chemical shifts in δ-scale). Mass spectra were recorded on Hitachi RMU-6E and Jeol JMS-300 instruments.

Extraction, isolation and separation of compounds. The dry bark powder (2 kg) of *Couropita guianeusis* was repeatedly extracted in a soxhlet apparatus with ethanol. The extract (10 L) was concentrated to a small volume (1L) and then fractionated into *n*-hexane, benzene and methanol solubles.

Compounds fdrom *n*-hexane extract. The reddish-brown hexane extract (2 L) was concentrated to a small volume (200 mL) and left for a few days at room temperature when a colourless solid, 200 mg (β-amyrin), separated out. The solvent from the mother liquor was removed and the concentrate absorbed on Si gel (100 g) and the material subjected to column chromatograpahy over Si gel eluting with hexane and hexane-benzene mixtures collectiang faractions of 800 mL each to furnish the following compounds. The hexane fractions 1-20 and hexane-benzene (9:1) fractions 21-30 furnished an oily fatty substance (300 mg).

β-Amyrin acetate. The hexane-benzene (8:2) fractions on evaporation left a solid which on crystallisation from chloroform-methanol gave colourless shining plates (50 mg), m.p. 240-42°; $[\alpha]_D$ +84° (*c* 1.0 in $CHCl_3$), identical in its physical and spectral characteristics with β-amyrin acetate[26].

β-Amyrone. The hexane-benzene (7:3) fractions on evaporation left a solid which on crystallisation from chloroform-methanol furnished colourless needles (50 mg), m.p. 175-76°; $[\alpha]_D$ +95° of β-amyrone, identified by comparison of its physical and spectral characteristics[6] and by direct comparison with an authentic sample.

β-Amyrin. The hexane-benzene (6:4) fractions 66-85 on evaporation furnished a solid which crystallised from methanol as long colourless needles (800 mg), m.p. 199-200°; $[\alpha]_D$ +94.2° (*c* 1.54 in benzene), identified as β-amyrin by comparison of its physical and spectral characteristics and by direct comparison with an authentic sample[6,8].

Mixture of β-sitosterol and stigmasterol. The hexane-benzene (1:1) column fractions 86-100 left a solid sterol mixture (200 mg) which was acetylated with pyridine and Ac_2O (5 mL each) and the acetate obtained on work-up was separated by preparative TLC over $AgNO_3$ impregnated Si gel into two compounds. The less polar compound, eluted by chloroform-methanol, crystallized as colourless needles (100 mg), m.p. 126-27°;$[\alpha]_D$ −38.2°, and was found identical in every respect with β-sitosteryl acetate. The more polar compound, eluted by chloroform-methanol, crystallized as colourless plates (50 mg), m.p. 142-43°;$[\alpha]_D$ − 42.3°, and was identified as stigmasteryl acetate.

Ergosta-4,6,8(14),22-tetraen-3-one[27]. The hexane-benzene (4:6) fractions 101-105 furnished a solid which crystallized from methanol as colourless needles, 80 mg, m.p. 80-81°;$[\alpha]_D$ +562° (*c* 1.1 in $CHCl_3$); R_f 0.76 (benzene-EtOAc, 4:1). Anal. Calcd for $C_{28}H_{40}O$: C,85.71; H, 10.20. Found: C, 85.66; H, 10.06%; UV (MeOH): 238, 282, 348 nm; IR (KBr): 1670, 1640, 1595, 968, 873, 760, 695 cm^{-1}.

^{1}H NMR (90 MHz): 0.81, 0.90, 0.96, 1.00, 1.10 (all methyls), 5.25 (2H, m, C_{22} and C_{23}-H), 5.74 (1H, s, C_4-H), 6.03, 6.60 (1H each, d, *J*=9Hz, C_6- and C_7-H); MS: m/z 392 (M^+, 18%), 364 (4), 349 (8), 321 (4), 268 (100), 267 (80), 253 (32), 250 (18), 214 (25), 173 (30), 124 (18). A comparison of the above data with those reported for ergosta-4,6,8(14), 22-tetraen-3-one proved its identity.

Couropitone, stigmasta-4,23-diene-3-one 1. The hexane-benzene (3:7) colouman fractions 106-115 on concentration left a solid which crystallised from methanol as colourless needles (20 mg), m.p. 103-04°; $[\alpha]_D$ +38.18° (*c* 0.16 in $CHCl_3$); R_f 0.38 (benzene -EtOAc, 4:1). Anal. Calcd for $C_{29}H_{46}O$: C, 84.88; H, 11.22. Found: C, 84.82; H, 11.12%; UV (MeOH): 241 nm; IR: (KBr): 2950, 1670, 1420, 1380, 1090, 980 and 970 cm^{-1}; ^{1}H NMR (90 MHz): 0.72 9 (3H, s, 18-H_3), 0.84 (3H, d, *J*= 7Hz, 21-H_3), 1.18 (3H, s, 19-H_3), 2.30 (2H, d, *J*=7 Hz, 28-CH_2), 5.10 (1H, m, C_{23}-H), 5.70 (1H, s, C_4-H); MS: m/z 420 (M^+, 20%), 367 (10), 298 (18), 283 (8), 271

(32), 269 (25), 229 (45), 175 (25), 124 (100), 107 (50), 95 (60).

2,4-Dinitrophenylhydrazone of stigmasta-4,23-diene-3-one. To couropitone (5 mg) in methanol (5 mL) was added 2,4-dinitrophenylhydrazine (10 mg) in methanol (5 mL) and a drop of conc. HCl. The mixture was warmed for 5 min. and left overnight to give an orange solid which crystallised from methanol as shining orange needles (4 mg), m.p. 142°.

Reduction of couropitone with $NaBH_4$: Isolation of stigmasta-4,23-diene-3β-ol 2. To couropitone (10 mg) in methanol (15 mL) was added $NaBH_4$ (20 mg) and the mixture refluxed for 6 hr on a water-bath. Usual work-up gave a solid which on initial purification by passing through a small column of Si gel and subsequent crystallisation from chloroform-methanol gave colourless needles of the alcohol **2** (8 mg), m.p. 132-33°; $[\alpha]_D$ +64.4° (*c* 0.86 in $CHCl_3$). Anal. Calcd for $C_{29}H_{48}O$: C, 84.46; H, 11.65. Found: C, 84.36; H, 11.6%; no UV absorption above 210 nm; IR (KBr): 3510, 2950, 1600, 1470, 1390 and 1030 cm^{-1}; ^1H NMR (90 MHz): 0.72, 0.84, 0.91, 1.00, 1.20 (all methyls), 2.30 (1H, m, 25-H), 4.20 (1H, br m, $W_{1/2}$ = 20 Hz, 3a-H), 5.10 (1H, m, 23-H), 5.30 (1H, d, J = 6 Hz, 4-H).

Compounds from benzene extract. The light brown benzene extract (2 L) on concentration left a residue (2 g) which was chromatographed over a column of Si gel. The hexane eluate gave an oil (100 mg). The hexane-benzene (4:1) eluate gave a gummy substance (100 mg). The hexane-benzene (1:1) fractions gave a solid (150 mg), m.p. 135-36°;$[\alpha]_D$ −35°, which gavė +ve LB test (violet →blue→green) for steroids and was identified as β-sitosterol. The hexane-benzene (4:6) eluate gave a solid (30 mg), m.p. 80-81°, which was found identical in every respect with with those of ergosta-4,6,8(14),22-tetraen-3-one obtained from the hexane extract.

Compounds from methanol extract. The reddish-brown methanolic extract (2 L) was concentrated to a small volume (200 mL) and treated with basic lead acetate to remove tannins and other phenolic substances. The tannin free and deleaded extract on concentration left a solid (1.5 g) which on chromatography over a column of Si gel and eluting with chloroform-methanol (95:5) left a solid which on crystallisation from chloroform-methanol gave colourless shining plates (150 mg), m.p. 282-84°; $[\alpha]_D$ −40° (*c* 0.6 in pyridine). The solid gave +ve LB test for steroids and positive Molish test for glycoside. It was found identical in every respect with β-sitosterol-3β(+)-D-glucoside. No more useful compound was obtained from the column.

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