
Extraction, Isolation and Identification of Two Prenylated Isoflavans From *Diatium Dinklagei* (Leguminosae)

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Abstract

7,2',4' - trihydroxy-6-(1'',2''-dimethyl-allyl) isoflavan (1) and (7,2',4'-trihydroxy-6-(1''-methyl-1''-hydroxymethyl-allyl) isoflavan (2) have been isolated from D. dinklagei (Harns (Leguminosae) and their structures determined from spectral data. Both compounds showed moderate bactericidal activity.

Introduction

Such compounds as rotenoids, flavonoids and isoflavonoids have been isolated from various species of this family (Harborne, 1982; Khalid and Waterman, 1983). Debarked stems of *Milletia recemosa* (Leguminosae) have yielded some flavonoid compounds (Khalid and Waterman, 1983; Prakash and Kupradanam, 1994). *Diatium* finds application in African folk medicine which has given the impetus to the investigation of its stem for potent medicinal compounds.

Results and Discussion

HRMS (High Resolution Mass Spectrometry) of compound **1** indicated its molecular formula to be C₂₀H₂₂O₄. Its IR Spectrum displayed bands for hydroxyl group, 3600 and 3450cm⁻¹. The bands, in the UV spectrum at 225(4.19), 282(3.39), and 287(3.92)nm were characteristic of isoflavans (Harborne, 1982). Compound **1** formed a triacetate and a trimethylether indicating the presence of three hydroxyl groups. The ¹H NMR spectrum of **1** showed isoflavan heterocyclic protons at δ 4.30(IH, bd), 3.95(IH, t), 3.50(IH, m) and 2.90(2H, m) and signals at δ 1.38 (3H, d), 4.15(IH, q), 1.68(3H, s) 5.02 (2H, d) which were assignable to a 1, 2 dimethylallyl group which has, hitherto, not been known in isoflavans. The presence of this unusual group in compound **1** was confirmed from ¹³C NMR data, as follows: 18.7 (C-1'', CH₃), 21.0 (C-2'', CH₃), 40.8 (C-1'') 122.7 (C-3'') and 150.1 (C-2''). The MS of **1** showed peaks at ^{m/z} 326 [M]⁺, 191 and 136 (RDA fragments).

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Based on the above spectral evidence, the structure of 1 was formulated as shown.

The molecular formula of 2 was $C_{20}H_{22}O_5$ (HRMS) and it formed a tetraacetate and tetramethylether indicating the presence of four hydroxyl groups. UV bands in 2 were observed at 222(4.18), 284(3.92) and 288(3.92)nm. 1H NMR showed the heterocyclic protons of isoflavans at δ 3.92 (1H,t, J = 10, 10Hz, H-4ax), 4.20 (1H,dd, J = 10, 3Hz, H-2eq), 3.42 (1H,m, H-3ax) 2.95 (1H,dd, J = 15.7, 10.5Hz, H-2ax), 2.75 (1H,t, J = 15.7 5.1Hz, H-4eq) – IR and 1H NMR spectral data ($3510cm^{-1}$, δ 3.75,3.90) also revealed the presence of a CH_2OH group which was part of a prenyl group (1''-hydroxymethyl-1''-methylallyl). The presence of a C_5 unit in isoflavan is reported for the first time. ^{13}C NMR peaks at δ 22.2, 46.1, 69.6, 113.1 and 144.7 could be assigned to this novel C_5 unit. In the MS of 2 fragment ions at m/z 207, 189 and 175 confirmed the presence as indicated.

Experimental

Air-dried debarked stems of *D. Dinklagei* collected from Ntan Obu, Eniong Abatim, Odukpani, L.G.A were powdered [5kg] and extracted with MeOH (10L). Concentration of the extract yielded a dark coloured semi solid (60kg) which was extracted with Et_2O and the concentrated Et_2O soluble portion (40g) chromatographed over silica gel eluting with varying proportions of $CHCl_3$: EtOAc mixture: Fraction eluted with (4:1) eluent mixture yielded 1 while fraction with (3:2) eluent mixture yielded 2.

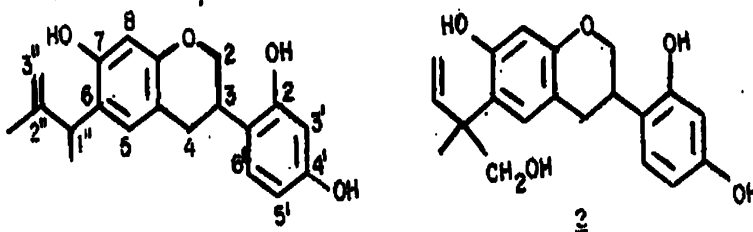
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Compound 1: Semi solid (250mg). (found: C, 73, 65 H, 6.77; $C_{20}H_{22}O_4$ requires C, 73.62, H, 6.75%), $[\alpha]_D^{25} -6^{\circ}$ (c 0.1, MeOH), HRMS: m/z 326, 1622; EIMS: m/z 326 [M]⁺, 311, 191 (base peak), 175, 136, 123, 115, 107, 91, 77, 69, 55; UV λ_{max}^{MeOH} (nm) (log ϵ), 225 (4.19), 282 (3.93), 287 (3.92). ¹H NMR (200 MHz, CDCl₃): δ 83.95 (1H,t,J=10Hz, H-2ax), 4.30 (1H,bd, J=10Hz, H-2eq), 3.50(1H, m, H-3ax); 2.90 (2H, m, H-4), 6.78 (1H, s, H-5), 6.30-6.35 (3H,m, H-8' and H-5'). 6.90(1H, d, J=10Hz, H-6'), 4:15 (1H, q, 4-1"), 1.38 (3H,d, J=8Hz, CH₃-1"), 1.68(3H, s, CH₃-2"), 5.02 (2H,d, J=12 Hz, H-3"). ¹³C NMR (200MHz, CDCl₃): δ 69.9 (C-2), 31.7 (C-3), 30.9 (C-4), 114.6 (C-4a), 129.0 (C-5), 122.7 (C-6), 155.3 (C-7), 103.7(C-8), 153.1(C-8a), 119.8(C-1'), 154.8 (C-3'), 153.2 (C-4'), 17.6(C-5'), 128.1(C-6), 40.8(C-1"), 150.1(C-2"), 122.7(C-3"), 18.7(C-1", CH₃), 21.02 (C-2", CH₃); CD (MeOH): $[\theta]_{287} + 1.239 \times 10^3$

Compound 2: Mp 118^o (500mg). (Found: C, 70.19; H, 6.42, $C_{20}H_{22}O_5$) requires C 70.18; H, 6.43%. $[\alpha]_D^{25} - 30$ (c 0.13, MeOH). HRMS: m/z 342.1312; EIMS: m/z 342 [M]⁺, 311, 285, 207, 189, 175, 149, 136, 123 (base peak), 107, 91, 79, 63, 43; IR $\sqrt{\frac{KBr}{max}} cm^{-1}$: 3510,3350,2900,1620, 1120. UV (MeOH) λ_{max} nm (log ϵ) 222 (4.18), 284(3.92), 288(3.92); ¹H NMR (200 MHz acetone-d₆): δ 83.92(1H, t, J = 10Hz, H-2ax), 4.20 (1H, dd, J=10, H-2eq), 3.42(1H,m, H-3ax), 2.95(1H, dd, J= 15.7,10.5 Hz, H-4ax), 2.75 (1 H, dd, J = 15.7,5.HHz, H-4eq), 6.93 (I H, s, H-5), 6.28 (I H, s, I-I-8), 6.44 (I II, d, J =2.3Hz, H-3'), 6.32 (1 H, dd, J = 2.3, 9Hz, H-5'), 6.99 (III, d, J = 9Hz, H-6'), 6.25 (IH, dd, J= 10.6, 17.7Hz, 11-2"), 5.05 (21-1, dd, τ = 17.1, 1.1Hz, H-3"), 1.40 (3H, s, CH₃-1 "). ¹³C/NMR (200MHz, acetone -d₆): 070.0 (C-2), 32.1 (C-3), 30.7 (C-4), 112.2 (C-4a), 129.8 (C-5), 123.7 (C-6), 157.2 (C-7), 104.5 (C-8), 155.1 (C-8a), 118.9 (C-1'), 154.0 (C-2'), 102.8 (C-3'), 156.1 (C-4'), 106.9 (C-5'), 128.0 (C-6'), 46.1 (C-1"), 144.7 (C-2"), 113.1 (C-3"), 22.2 (C-1 CH₃), 69.56 (C-1", Cl12 OH); CD(MeOH): $[\theta]_{288}+1.3X 10^3$

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These compounds demonstrated selective toxicity to Gram-positive bacteria (*Staphylococcus aureus*) at $0.1 \mu\text{g mL}^{-1}$



References

- Harbone, J. B. & Mabry, T. J. (1982) *Advances in research: The Flavonoids*, Chapman Hall, New York, p.536
- Ingham, J. L. (1997). The structure of Neovestitol, a new isoflavan. *Z. Naturforsch*, 34c, 630-632
- Ingham, J. L. (1997). Isoflavan phytoalexins from *Anthyllis lotus* and *tetragonolobus*. *Phytochemistry* 16, 1279-128
- Jadish-Kumar, R, Krupadanam, G. L. D. & Srimannarayana, G. (1989). Flavonoids from *Milletia racemosa*. *Phytochemistry*, 28, 913-1916
- Khalid, S. A. & Waterman, P. G. (1983). Flavonoids, isoflavanoid and rotenoid compounds from leguminosae. *Phytochemistry*, 22, 101-104
- Prakash R.A O, C. & Kupradanam. G. L. D. (1994). Biosynthesis of Iridoid glycoside in *Thunbergia alata*. *Phytochemistry*, 35, 1597-1600