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Two aurone glycosides from heartwood of *Pterocarpus santalinus*

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Abstract

Two aurone glycosides, 6 hydroxy 5 methyl 3′,4′,5′ trimethoxy aurone 4-*O*-α-L-rhamnopyranoside and 6,4′ dihydroxy aurone 4-*O*-rutinoside have been isolated from the ethanolic extract of the wood of *Pterocarpus santalinus*. Their structures were determined on the basis of chemical and spectroscopic analysis (UV, IR, EIMS, ^{1}H and ^{13}C NMR).

Keywords: *Pterocarpus santalinus*; Papilionaceae; Red Sandalwood, Aurone glycosides; 6 hydroxy 5 methyl 3′,4′,5′ trimethoxyaurone-4-*O*-α-L-rhamnopyranoside; 6,4′ dihydroxy aurone-4-*O*-rutinoside

1. Introduction

Many species of Pterocarpus are known. These species are found to be rich in isoflavonoids (Mitra and Joshi, 1983), terpinoids and related phenolic compounds (Kumar et al., 1974; Kumar and Seshadri, 1976; Seshadri, 1972), β-sitosterol (Ivorra et al., 1989), lupeol (Harborne and Barter, 1983), (−) epicatechin (Chakravarthy and Gode, 1985) and aurone glycosides (Mohan and Joshi, 1989). Aurones occurs rarely in nature and only a limited number of aurones has been isolated from plant source. Aurones are important contributor to pigmentation of the flowers by virtue of their bright golden yellow colour. A series of naturally occurring aurones have been reported to exhibit antiplasmodial activity (Kayser et al., 2001) and has been used as a potential antileishmanial drug (Kayser et al., 1998). From the ethanolic extract of the heartwood of *Pterocarpus santalinus* (Fam.:Papilionaceae), commonly known as Red Sandalwood, two new aurone glycosides have been isolated and their structures were elucidated on the basis of chemical and spectral studies.

2. Result and discussion

Compound I, $C_{25}H_{28}O_{12}$, was found to be a non-reducing glycoside as it gives positive Molisch test and a negative aniline hydrogen phthalate test. IR studies of the compound showed prominent peaks at 3410 cm^{-1} (OH), 2945 and 1160 cm^{-1} (methoxyl), 1635 cm^{-1} (> C═O), 2930 and 1452 cm^{-1} (C–CH_3) and 1281 cm^{-1} (–C–O–C– vibration). On acid hydrolysis (7% H_2SO_4) it gave an aglycone and a sugar, identified as rhamnose by paper co-chromatography with authentic sample and by ^{1}H NMR spectral analysis of the glycoside (a doublet at 1.10 corresponding to three protons of rhamnosyl –Me group, broad signal at δ 3.5–3.8 for four protons and a singlet at δ 4.2 due to C-1″ protons of rhamnose).

The aglycone, $C_{19}H_{18}O_7$ was found to be aurone on the basis of colour reaction (Jurd, 1962) and UV spectral data (Mabry et al., 1970). Aglycone was analysed for three methoxyl groups and their presence was confirmed by a singlet at δ 3.63 (9H). NMR showed presence of

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methoxyl groups at 3′,4′ and 5′ position. ^{1}H NMR studies of the aglycone showed the presence of three aromatic protons suggesting a hexasubstituted aurone. A multiplet at δ 7.88–8.00 (2H) was due to C2′ and C6′ protons and a singlet at δ 6.23 (1H) for C7 protons. A singlet at δ 1.52 (3H) and δ 6.75 (1H) was assignable to 3H of –Me group and benzyne proton (=CH–) (Nascimento et al., 1976), respectively. The above position of various groups were further confirmed by ^{13}C NMR spectral analysis.

On acetylation aglycone formed diacetate, m.p. 104°, indicating the presence of two hydroxyl groups. Mass spectral data showed a molecular ion peak at 520. Two fragments of *m*/*z* 192 and 166 showed that three methoxyl groups were present in the ring B and two hydroxyl and one methyl group in the ring A. The positions of hydroxyls were shown to be C4 and C6 of aglycone by UV spectral shifts (Mabry and Markham, 1975). The only positions left were C-5 and C-7 for attachment of methyl group in the ring A. C5 position for methyl group was confirmed by ^{1}H NMR.Values from ^{13}C NMR, also point to a structure with ring A identical to the 5 methyl aurone (Seabre et al., 1995; Seabre, 1997).

Attachment of the sugar at C4 of the aglycone was determined by comparing the ^{13}C NMR spectra of the glycoside with that of aglycone. The C4 of the glycoside in ^{13}C NMR spectra appeared at downfield (162.1) than C4 of the aglycone (160.8). No change in λ_{max} with the addition of $AlCl_3$ confirmed that Compound I was a 4-*O*-glycoside (Harborne, 1967).

Quantitative sugar estimation suggested the glycoside to be a monosaccharide. Consumption of 2 mole of periodate by the glycoside and consequent liberation of 1 mole of formic acid confirmed the pyranose form of sugar. The hydrolysis of the glycoside with takadiastase confirmed the α nature of the glycoside linkage. This led to the formulation of the Compound I as 6 hydroxy 5 methyl 3′,4′,5′ trimethoxy aurone 4-*O*-α-L-rhamnopyranoside (see Fig. 1).

Compound II, $C_{27}H_{30}O_{14}$, was also found to be a non reducing glycoside. On acid hydrolysis (7% H_2SO_4) it gave an aglycone and two sugars. The sugars were identified as rhamnose and glucose by co-chromatography

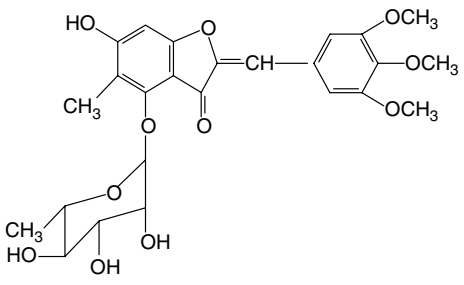

Fig. 1. 6 Hydroxy 5 methyl 3′,4′,5′ trimethoxyaurone 4-*O*-α-L-rhamnopyranoside.

with authentic samples and NMR studies. No change in λ_{max} with the addition of $AlCl_3$ showed that compound II was a 4-*O*-glycoside (Harborne, 1967).

^{1}H NMR spectra of the glycoside showed a broad signal at δ 0.92 ppm corresponding to methyl protons of the rhamnose. A broad signal at δ 3.50–3.85 ppm was due to 10 H sugar protons. A singlet at δ 4.9 ppm and a broad band at δ 5.27 ppm corresponds to rhamnose C1‴ proton and glucosyl C-1″ protons, respectively. The ^{1}H NMR signal of rhamnose methyl (3H, br, J = 7 Hz) at 0.92 showed the presence of 1 → 6 intersugar linkage (rutinoside moiety) in the glycoside, since this signal commonly appears as a doublet at δ 1.2–1.35 ppm in neohesperidosides and as a broad singlet at δ 0.80–0.95 ppm in rutinoside (Kutney et al., 1970; Sherwood et al., 1973; Gupta et al., 1989).

Aglycone, $C_{15}H_{10}O_5$ was found to be aurone on the basis of colour reaction (Jurd, 1962) and UV spectral data (Mabry et al., 1970). IR data clearly indicate the presence of hydroxyl (3370 cm^{-1}) and carbonyl group (1631 cm^{-1}) in the compound. ^{1}H NMR studies of the aglycone showed the presence of six aromatic protons suggesting a trisubstituted aurone. A doublet at δ 6.15 and δ 6.5 was due to C-5 and C-7 protons respectively. A multiplet at δ 7.75–7.9 (2H) was assigned to C2′ and C6′ protons and a doublet at δ 6.94 was due to C3′ and C5′ protons. A singlet at δ 6.70 ppm was assigned to benzyne proton (=CH–) (Nascimento et al., 1976). Values from ^{13}C NMR confirmed all presented datas.

Aglycone on acetylation formed triacetate indicating the presence of three-hydroxyl group. Mass spectral data showed a molecular ion peak at 578. The ion peak at *m*/*z* 152 and 118, indicate that two hydroxyl groups were in ring A and one hydroxyl group in ring B. The positions of hydroxyl were shown at C4 and C6 of aglycone by UV spectral shifts (Mabry et al., 1970) and ^{13}C NMR. In ring B, the presence of doublet at δ 6.94 (2H) for 3′ and 5′ indicate para position of OH in ring B. The downfield shift in ^{13}C NMR of C4′ confirmed OH position at C4′. The aglycone was identified as 4,6,4′ trihydroxyaurone. The aglycone has already been isolated from flowers of *Limonium* (Asen and Plimma, 1972).

The attachment of sugar moieties were confirmed by comparing the ^{13}C NMR spectral data of glycoside and aglycone which showed attachment of sugar at C4 by higher δ value at C4 for glycoside (δ 163.1) and lower δ value for aglycone (δ 161.4). In the disaccharide unit glucose and rhamnose was present in molar ratio 1:1 as indicated by paper co-chromatography. Rhamnose was found to be terminal sugar, as it appeared first during mild acid hydrolysis of the glycoside, followed by glucose.

The structure was finally established by permethylation of 1 followed by acid hydrolysis that gave 2,3,4-tri-*O*-methyl rhamnose and 2,3,4-tri-*O*-methyl glucose identified by R_G values (Mikes, 1967). This confirmed the (1 → 6) intersugar linkage and pyranose ring struc-

ture for both sugars as the hydroxyls at position 4 in both sugars were methylated.

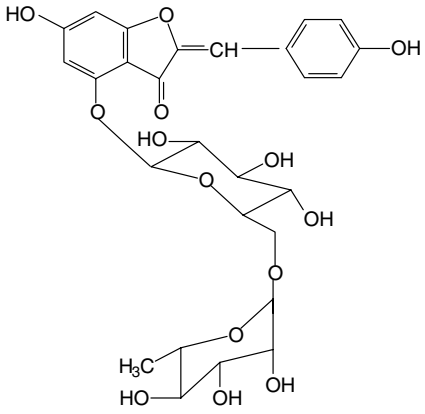

Fig. 2. 6,4′ Dihydroxyaurone 4-*O*-rutinoside.

The sterochemical nature of the inter-sugar as well as the glycosidic linkage was established by enzymatic hydrolysis. With takadiastase only Rhamnose was hydrolysed and when this partially hydrolysed glycoside was treated with emulsin it was completely hydrolysed. This indicate that inter sugar linkage is α and glycoside linkage as β in nature. On the basis of these evidences structure of Compound II was formulated as 6,4′ dihydroxy aurone 4-*O*-rutinoside (see Fig. 2).

3. Experimental

Four kilogram of crushed wood was extracted in boiling ethanol. The concentrated ethanolic extract was poured into ice-cold water whereby a aqueous solution and a coloured residue were obtained.

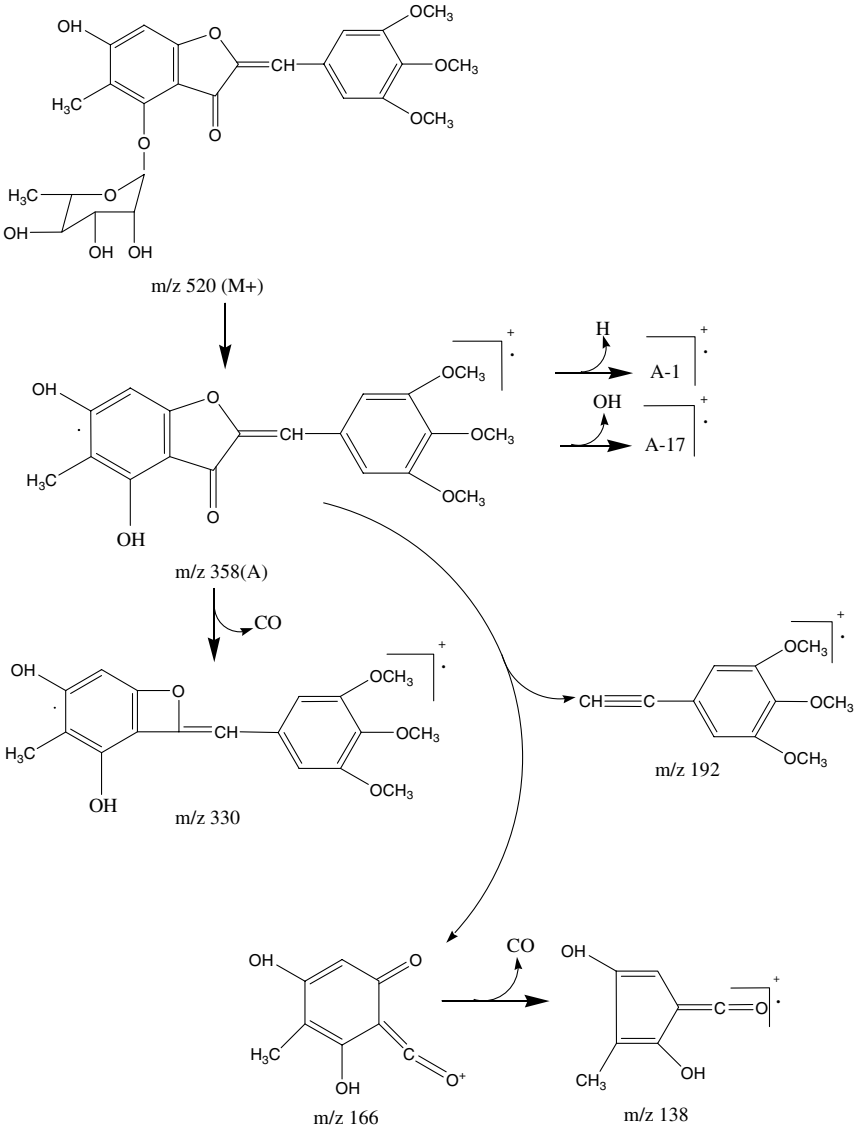

Fig. 3. Mass spectral fragmentation of Compound I.

The aqueous solution obtained was concentrated and was successively extracted with petroleum ether, benzene, ether and ethyl acetate. The ether fraction was chromatographed over silica gel column and eluted with hexane and then with benzene. A yellow colored compound was obtained from benzene: hexane (9:1). The compound was crystallized from methanol and identified as Compound I, $C_{25}H_{28}O_{12}$, m.p. 192°.

Elemental analysis: Found: C, 57.58%; H, 5.42%. Calculated C, 57.70%, H, 5.38%. UV λ_{max} nm: 260, 330, 405; +$AlCl_3$ 260, 330, 405; + NaOMe 262, 338, 468; +NaOAc 263, 336, 462; IR ν_{max}: 3410, 2945, 2930, 1635, 1452, 1281, 1160, 720, 670 cm^{-1}. NMR ($CDCl_3$, 100 MHz): δ 1.1(d,3H, rhamnose methyl), 1.63 (S, 3H, –CH_3), 3.5–3.8 (br, 4H, sugar protons), 3.65 (S, 9H, –OCH_3), 4.2 (S, 1H, H1″), 6.23 (S, 1H, H-7), 6.72 (S, 1H, = CH–), 7.88–8.00 (m, 2H, H2′ and 6′) ppm. C^{13} analysis: C2 δ 154.4, C3 δ 190.4, C4 δ 162.1, C5 δ 120.4, C6 δ 161.8, C7 δ 94.6, C8 δ 160.6, C9 δ 106.4, C10 δ 114.2, C-1′ δ 122.1,C2′ and C6′ δ115.6, C3′ and C5′ δ159.7, C4′ δ154.2, –CH_3 23.8, O–CH_3 δ 58.6, C-1″ δ 101.4, C2″ δ 72.6, C3″ δ 72.3, C4″ δ 73.7, C5″ δ 70.4, C6″ δ 18.8. MS (70 eV) *m/z* 520 (M^+), 358, 357, 341, 330, 192, 166, 138 (see Fig. 3).

Acid Hydrolysis (7% H_2SO_4) of Compound I yielded an aglycone and rhamnose. Aglycone of I, $C_{19}H_{18}O_7$, m.p. 212°. Elemental analysis: Found: C, 63.64%; H, 5.15%. Calculated C, 63.68%, H, 5.03%. UV λ_{max} nm: 262, 328, 408, +$AlCl_3$ 260, 335, 452, +NaOMe 262, 338, 440, +NaOAc 260, 336, 442, NMR ($CDCl_3$, 100 MHz): δ 1.52 (S, 3H, –CH_3), δ 3.63 (S, 9H, –OCH_3), δ 6.23 (S, 1H, H-7), δ 6.75 (S, 1H, =CH–), δ 7.88–8.00 (m, 2H, H2′ and 6′) ppm. C^{13} analysis: C2 δ 154.2, C3 δ 190.0, C4 δ 160.8, C5 δ 120.2, C6 δ 161.7, C7 δ 94.8, C8 δ 160.6, C9 δ 106.2, C10 δ 114.2, C-1′ δ 122.2,C2′ and C6′ δ115.6, C3′ and C5′ δ159.8, C4′ δ154.2, –CH_3 23.5, O–CH_3 δ 58.6. MS (70 eV) *m/z* 358 (M^+), 357, 341, 330, 192, 166, 138.

Acetate of aglycone I (pyridine–Ac_2O, 24 hours at room temperature), m.p. 104° Elemental analysis: Found: C, 63.66%; H, 4.92%; acetyl, 22.45%. Calculated C, 63.75%; H, 4.80%; acetyl, 22.27%.

The ethyl acetate fraction of aqueous solution was chromatographed over silica gel column and eluted suc-

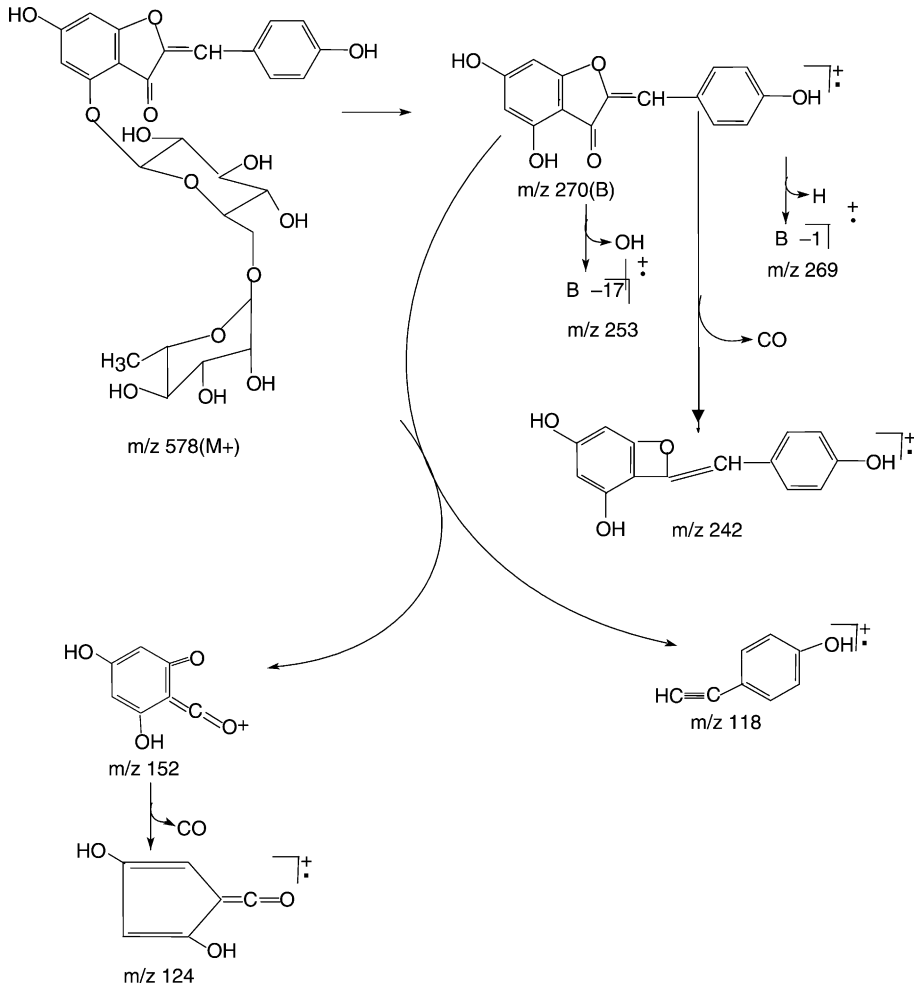

Fig. 4. Mass spectral fragmentation of Compound II.

cessively with solvents of increasing polarity. Compound II, $C_{27}H_{30}O_{14}$, m.p. 228°, was obtained from ethyl acetate eluate and this yellow colored compound was crystallized from MeOH.

Elemental analysis: Found: C, 56.20%; H, 5.35%. Calculated C, 56.05%; H, 5.20%. UV λ_{max} nm: 260, 340, 398. +$AlCl_3$ 260, 340, 398, +NaOMe 262, 348, 456, +NaOAc 260, 344, 458 nm. IR ν_{max}: 3370, 1631, 1268, 730, 680 cm^{-1}. NMR ($CDCl_3$, 250 MHz): δ 0.92 (br, 3H, $J = 7$ Hz, rhamnose methyl), 3.50–3.85(m, 10H, sugar protons), δ 4.9 (S, 1H, H-1″, rhamnosyl), 5.27 (br, 1H, H-1″ glucosyl) 6.2(d, 1H, $J = 2$ Hz, H-5) 6.5 (d, 1H, $J = 2$ Hz, H-7), 6.70 (S, 1H, =CH–), 7.7–7.9 (m, 2H, H2′ and 6′), 6.9 (d, 2H, H3′ and H5′) ppm. C^{13} analysis: C2 δ 146.2, C3 δ 192.4, C4 δ 163.1, C5 δ 92.4, C6 δ 160.3, C7 δ 97.4, C8 δ 160.8, C9 δ 103.2, C10 δ 112.8, C-1′ δ 121.8, C2′ and C6′ δ129.2, C3′ and C5′ δ114.2, C4′ δ160.2, C-1″ δ 104.8, C2″ δ 75.1, C3″ δ 78.2, C4″ δ 71.4, C5″ δ 78.2, C6″ δ 67.5, C1‴ δ 101.2, C2‴ δ 72.2, C3‴ δ 72.5, C4‴ δ 73.7, C5‴ δ 70.2, C6‴ δ 18.5. MS (70 eV) m/z 578 (M^+), 270, 269, 253, 242, 152, 124, 118 (see Fig. 4).

Hydrolysis: On acid hydrolysis, glycoside II, gave an Aglycone, $C_{15}H_{10}O_5$, m.p. 280°, and two sugars glucose and rhamnose. Elemental analysis: Found: C, 66.58%, H, 3.75%. Calculated C, 66.66%; H, 3.71%. UV λ_{max} nm: 225, 245, 345, 392 +$AlCl_3$ 260, 350, 390, 451, +NaOMe 262, 360, 444, +NaOAc 260, 344, 450. NMR ($CDCl_3$, 250 MHz): δ 6.15 (d,1H, H-5), δ 6.5 (d,1H, H-7), 7.75– 7.9 (m, 2H, H2′ and H 6′), δ 6.94 (d, 2H, H3′ and H5′), 6.70 (S, 1H, =CH–) ppm. C^{13} analysis: C2 δ 146.2, C3 δ 192.0, C4 δ 161.4, C5 δ 92.2, C6 δ 160.2, C7 δ 97.6, C8 δ 160.8, C9 δ 102.8, C10 δ 112.8, C-1′ δ 122, C2′ and C6′ δ129.2, C3′ and C5′ δ114.4, C4′ δ160.3. MS (70 eV): m/z 270 (M^+), 269, 253, 242, 152, 124, 118.

Acetate of aglycone II (pyridine–Ac_2O, 24 h at room temperature), m.p. 184° Elemental analysis: Found: C, 63.56%; H, 4.12%; acetyl, 32.80%. Calculated C, 63.63%; H, 4.04%; acetyl, 32.57%.

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