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DALBIN: A 12*a*-HYDROXY ROTENOID GLYCOSIDE FROM *DALBERGIA LATIFOLIA*

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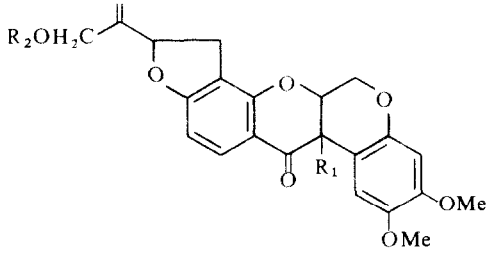
Key Word Index—*Dalbergia latifolia*; Leguminosae; seeds; dalbin; rotenoid; (−)-12*a*-hydroxyamorphigenin-8-*O*-*β*-D-glucopyranoside.

In continuation of our work on *Dalbergia latifolia* seeds [1], we report in this paper the isolation and structure of a new rotenoid glycoside, dalbin obtained from an alcohol extract.

The defatted EtOH extract of seeds was treated with MeOH which gave (a) MeOH insoluble solid and (b) MeOH soluble part. The latter was concentrated and the residue fractionated into (i) EtOAc insoluble semisolid and (ii) EtOAc soluble part. The EtOAc soluble part was concentrated to a small volume and precipitated with Et_2O. The Et_2O insoluble white solid was chromatographed on a Si gel column using EtOAc as solvent. The fractions so obtained on concn yielded the new rotenoid glycoside named as dalbin. It analysed for $C_{29}H_{32}O_{13}$ had mp 161–163°; $[\alpha]_D$ −58.80 (*c*, 0.17, MeOH) and gave positive Durham's and Molisch's tests suggesting that it was a rotenoid glycoside. ν_{max}^{KBr} cm^{-1}: 3490 (—OH) and 1675 (12-carbonyl) cm^{-1}. λ_{max}^{MeOH} nm (log ε): 235 (4.03), 245 *sh*(3.91) and 295 (4.14).

Dalbin on acid hydrolysis gave D-glucose as the only sugar and one major pale-yellow compound, mp 226° (d). ν_{max}^{KBr}: 3460 (—OH), 1634 (12-carbonyl) cm^{-1}; λ_{max}^{MeOH} nm (log ε): 235 (4.34), 280 (4.25) and 310 (4.17). It was identified as 6*a*, 12*a*-dehydroamorphigenin by comparison with the literature [2]. Thus the formation of 6*a*, 12*a*-dehydro product in this process indicated the amorphigenin (**1**) skeleton for the parent glycoside.

On enzymatic hydrolysis with almond emulsin (*β*-glucosidase), dalbin gave D-glucose and dalbinol [1] (**2**), identified by direct comparison with the natural authentic sample isolated from the same source. These observations indicated that the parent glycoside is a glucoside of dalbinol. Permethylation of dalbin by Hakomori's method followed by Killiani's hydrolysis gave 2,3,4,6-tetra-*O*-methyl-D-glucose as the only methylated sugar indicating the pyranose form for the glucose residue.



(**1**) $R_1 = R_2 = H$
(**2**) $R_1 = OH$; $R_2 = H$
(**3**) $R_1 = OH$; R_2 = *β*-glucosyl

Dalbin (0.05 g) like dalbinol on warming with conc H_2SO_4 (0.25 ml) and HOAc (2 ml) gave a dark yellow compound, mp 177–178°; ν_{max}^{KBr} cm^{-1}: 1739 (acetate), 1634 (12-carbonyl); λ_{max}^{MeOH} nm (log ε): 235 (4.36); 280 (4.26), 310 (4.17); PMR (60 MHz, $CDCl_3$, TMS int. standard): δ 2.15 (3H, *s*, aliphatic —OAc), 3.82, 3.91 (2 × 3H, each *s*, 2 × —OCH_3), 3.4 (2H, *m*, C-4′), 4.71 (8′-methylene protons), 4.94 (2H, *s*, C-6), 5.29, 5.38 (2H, C-7′), 5.36 (1H, *m*, C-5′), 6.44 (1H, *s*, C-4), 6.82 (1H, *d*, $J_{10,11}$ = 9 Hz, C-10), 7.92 (1H, *d*, $J_{10,11}$ = 9 Hz, C-11) and 8.30 (1H, *s*, C-1). It was identified as 8′-acetoxy-6*a*, 12*a*-dehydroamorphigenin on chemical and spectral comparison with reported data [2]. The formation of 6*a*, 12*a*-dehydroamorphigenin acetate on acid catalysed dehydration further confirmed the structural similarity of the compounds dalbinol and dalbin.

Dalbin on acetylation formed a pentaacetate, mp 141–142°. PMR of dalbin acetate (60 MHz, $CDCl_3$, TMS as int. standard) δ 2.04 (4 × 3H, aliphatic —OAc), 2.14 (3H, *s*, aliphatic —OAc), 3.35 (2H, *m*, C-4′), 3.77, 3.85 (2 × 3H, each *s*, 2 × —OCH_3), 4.14–5.08 (*m*, 6, 6*a*, 8′-methylene and glucosyl protons), 5.15, 5.28 (2H, C-7′), 5.45 (1H, *m*, C-5′), 6.43 (1H, *s*, C-4), 6.51 (1H, *d*, $J_{10,11}$ = 9 Hz, C-10), 6.84 (1H, *s*, C-1), 7.88 (1H, *d*, $J_{10,11}$ = 9 Hz, C-11). The presence of 4 aliphatic acetoxyls at δ 2.04 confirmed the parent glycoside as a monoglucoside. The acetoxyl at δ 2.14 was attributed to the 12*a*-acetoxyl. The distinguishing feature of the PMR spectrum was the splitting of the 7′-vinylic protons into a doublet at δ 5.15 and 5.28 (J = 13 Hz). Amorphigenin and dalbinol show unresolved 7′-proton bands at δ 5.27 and 5.18, respectively; both of them on acetylation show 7′-vinylic splitting at δ 5.14, 5.22 (J = 8 Hz) and 5.16, 5.22 (J = 6 Hz), respectively. This splitting is caused by the long range shielding of the acetyl which produces a different chemical environment for the two vinyl protons. The large coupling constant for the 7′-doublet observed in dalbin acetate indicated the position of the glucose moiety at 8′.

That the 12*a*-hydroxyl is free in the parent glycoside is shown by the PMR of its acetate. In dalbinol, 1 and 4-proton signals which appear at δ 6.56 and 6.31, respectively became merged with the 10-proton doublet at δ 6.42. However, in dalbinol diacetate a 1-proton singlet appears distinct at δ 6.79 due to its downfield shift. The same downfield shift is observed in dalbin acetate at δ 6.84. This confirms the 8′-position of the glucose residue.

Thus dalbin can be represented as (−)-12*a*-hydroxyamorphigenin-8′-*O*-*β*-D-glycopyranoside (**3**). The assigned structure is supported by MS which showed a peak at *m/e* 208 as the base peak, a diagnostic feature of 12*a*-hydroxy rotenoids having a 2,3-dimethoxychroman system [3].

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METABOLISM OF HYGRINE IN *ATROPA*, *HYOSCYAMUS* AND *PHYSALIS*

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Key Word Index—*Physalis alkekengi*; *Atropa belladonna*; *Hyoscyamus niger*; Solanaceae; biosynthesis; tropane alkaloids; cuscohygrine; D(+) and L(−)-hygrine precursors.

Abstract—Three-month-old plants of *Physalis alkekengi, Atropa belladonna* and *Hyoscyamus niger* were fed via the roots with either D(+) or L(−)-hygrine-[2′-^{14}C]. The feeding experiments were terminated after 7 days when the following alkaloids were isolated from the paired groups of plants: tigloidine, 3α-tigloyloxytropane and cuscohygrine from *Physalis*; hyoscyamine from *Hyoscyamus* and from *Atropa*. In contrast to *Datura* these genera appear to use both hygrine enantiomers in the biosynthesis of the tropane ring.

We have recently reported [1] that D(+) (**5**) but not L(−)-hygrine (**6**) is a precursor in the biosynthesis of the tropane ring of the tropoyl esters hyoscine and hyoscyamine, and the tigloyl esters 3α,6*β*-ditigloyloxytropane and 3α,6*β*-ditigloyloxytropan-7*β*-ol in *Datura innoxia*. In view of the stereoselectivity demonstrated, it seemed appropriate to examine other members of the Solanaceae to discover whether this is a general consideration or one peculiar to *Datura*. According to Wettstein [2], the Solanaceae may be divided into 5 tribes, and plants containing tropane alkaloids occur in all except the Cestreae. The largest tropane alkaloid bearing tribe is the Solaneae and for this investigation representatives from 3 of its 4 subtribes were chosen: *Atropa* from the subtribe Lyciinae, *Hyoscyamus* from the Hyoscyaminae and *Physalis* from the Solaninae.

Table 1. Specific activities of alkaloids isolated from *Physalis alkekengi*, *Atropa belladonna* and *Hyoscyamus niger*

	Hygrine-[2′^{14}C] isomer fed	Alkaloid isolated	Sp. act. dpm/mmol × 10^{-5}*
Physalis alkekengi	(+)	(**1**)	0.50
	(−)	(**1**)	0.68
	(+)	(**2**)	3.82
	(−)	(**2**)	2.65
	(+)	(**3**)	2.13
	(−)	(**3**)	2.04
Atropa belladonna	(+)	(**4**)	0.63
	(−)	(**4**)	0.61
Hyoscyamus niger	(+)	(**4**)	2.52
	(−)	(**4**)	2.69

(**1**)—3α-Tigloyloxytropane; (**2**)—3*β*-tigloyloxytropane; (**3**)—cuscohygrine; (**4**)—hyoscyamine.

*Since the activity of the precursor was 1.0×10^7 then this figure also coincides with the % specific incorporation calculated as [Sp. act. base (dpm/mmol)]/[Sp. act. precursor (dpm/mmol)] × 100.

Hygrine is widely distributed in nature [3, 4] and it frequently co-occurs with tropane alkaloids. When isolated the base displays no optical activity but this could be due to the facile racemization of the alkaloid during extraction. However, the enantiomers are easily resolved [5] and (+)-hygrine (**5**) is known to have the *R* configuration [6]. Hygrine-[2′-^{14}C] was synthesized and resolved by means of the D(+)-tartrate salt in which form it was administered in order to avoid possible racemization during the course of the experiment. The alkaloids listed in Table 1 were separated on pH 5.6 and 6.8 partition columns and isolated as the picrates [7, 8].

In contrast to our previous results in which (+)-hygrine proved to be a specific precursor of the tropane alkaloids in *Datura* [1], *Atropa, Hyoscyamus* and *Physalis* appear to be able to use either isomer equally well (see Scheme 1). Alternatively, it is possible that they are able to convert (−)-hygrine to the *dextro* isomer. This is a most unexpected finding as we had anticipated that all tropane alkaloid bearing genera would function in the same way. The apparent difference in *Datura* [1] cannot be attributed to poor transport of the *laevo* isomer since (−)-hygrine labels cuscohygrine to an extent which is well above the calculated incorporation of the (+) isomer impurity in the precursor and we conclude that both isomers reached the site of synthesis. Further experiments are in progress to confirm and to examine the extent to which this difference exists in the tropane alkaloid bearing plants of the Solanaceae.