Synthesis of the Pyranoisoflavonoid, Heminitidulan. Isoflavanoid and Rotenoid Glycosides from the Bark of *Dalbergia nitidula* Welw. *ex* Bak.

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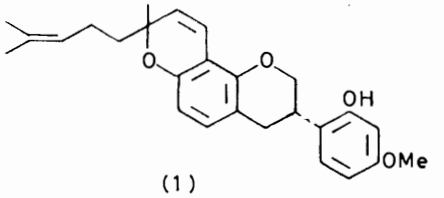
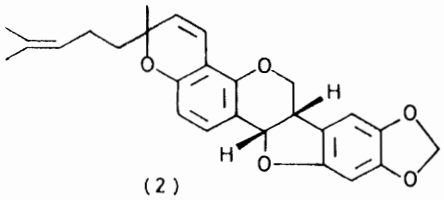
Synthesis of racemates of the complex pyranoisoflavan, (3*S*)-heminitidulan, a metabolite from the bark of *Dalbergia nitidula*, is accomplished. The isoflavone *C*-glycoside pairs 8-*C*-β-D-glucopyranosyl- and 6,8-di-*C*-β-glucopyranosyl-genistein and -orobol and the associated rotenoid glycoside (5′*R*,6a*R*,12a*R*)-8′-*O*-β-D-glucopyranosyl-12a-hydroxyamorphigenin comprise the predominant isoflavonoids in the same source. ^{13}C N.m.r. spectra of the glycosides are recorded.

BARK metabolites of *Dalbergia nitidula* Welw. *ex* Bak. comprise a mixture of eight complex (3*S*)-isoflavans and (6a*S*,12a*S*)-pterocarpans as well as a mixture of hitherto unidentified glycosides.[1] Members of both the above-mentioned groups of isoflavonoids all possess prenyl or geranyl side-chains, which, with one exception, are cyclized to stereochemically related 2*H*-pyran moieties.[1] Our previous synthesis of a derivative of a thus prenylated isoflavan, leiocin, is now extended to direct introduction of a geranyl side-chain and its cyclization to give heminitidulan (1). Similar attempts are made to synthesise a pterocarpanoid analogue, nitiducarpin (2).



(1)



(2)

The complete synthesis of heminitidulan (1) (*cf.* Scheme 1) follows the general lines previously outlined for leiocin,[1] except a completely different strategy is used for the introduction and removal of protective groups at each step. In the penultimate step an equivalent of the expected derivative of the ' linear ' isomer (11) of heminitidulan is formed from electrophilic 6- instead of 8-substitution of the 7-hydroxy-4′-methoxy-2′-phenacyloxyisoflavan (10) with citral.

Cyclization during the penultimate step introduces a second chiral centre, thus leading to two racemates for each of the isomers (11) and (12), and ultimately for synthetic heminitidulan. These pairs of racemates are not separable, the only indication of their presence being very narrowly split 2″-methyl resonances in the ^1H n.m.r. spectra of compound (12) and the synthetic heminitidulan derived directly from it by dephenacylation. By comparison the 2″-methyl resonances of natural heminitidulan [1] and of the ' linear ' synthetic isomer (11) of compound (12) are observed as singlets at 100 and 80 MHz respectively.

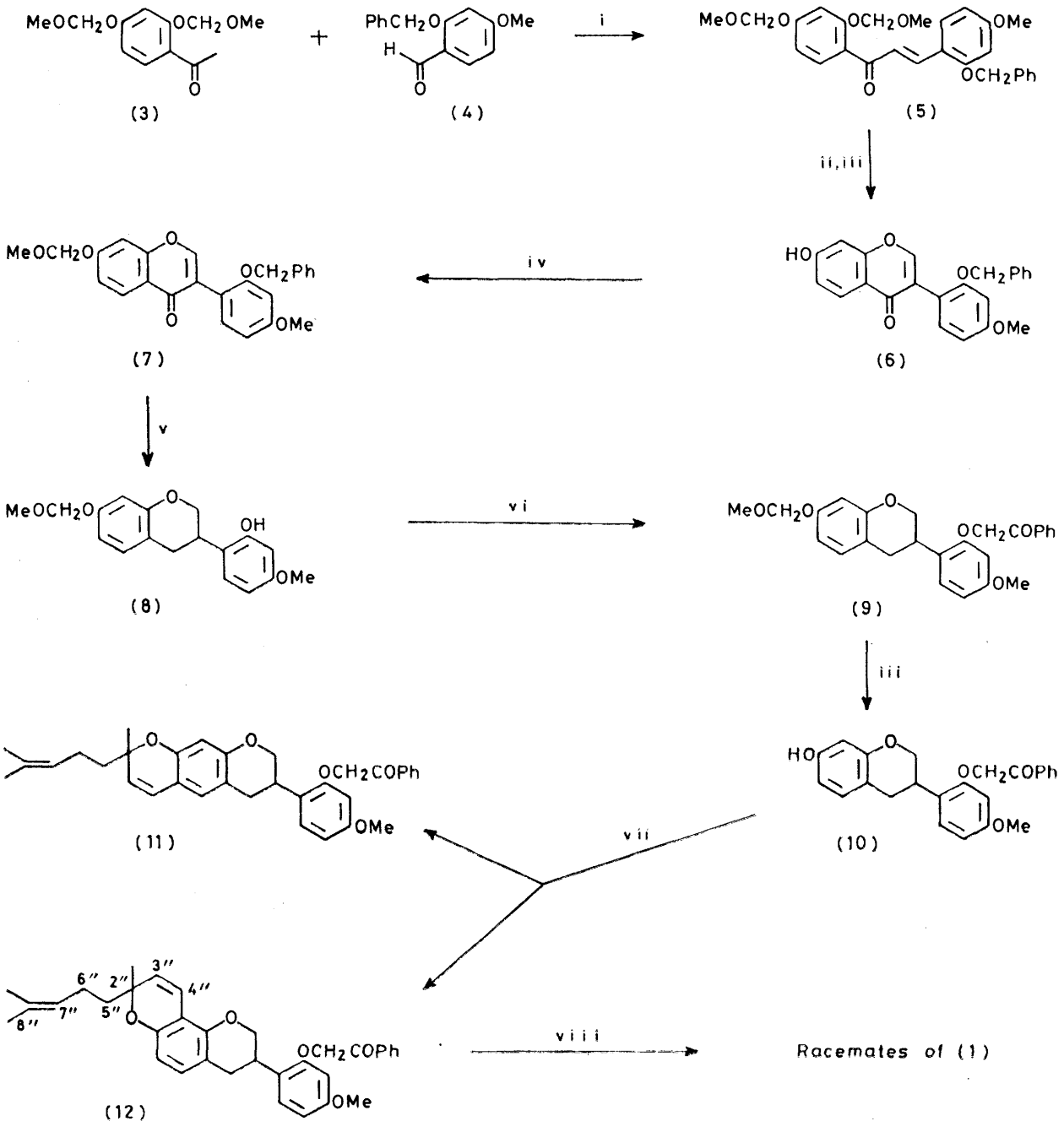
In contrast, attempted synthesis of nitiducarpin [1] (2) by direct introduction of the identical side-chain into synthetic [2] 3-hydroxy-8,9-methylenedioxypterocarpan [(15), maackiain [3]] met with no success after repeated attempts (*cf.* Scheme 2). An obvious alternative route *via* 2′,7-dihydroxy-4′,5′-methylenedioxyisoflavone (14) appeared promising in that it permitted introduction of the side-chain (16) prior to the proposed pterocarpan formation by reductive cyclization. However, the latter normally successful step [*cf.* (14) ⟶ (15)] used in the synthesis [2] of the pterocarpan gave a complex mixture of products. The reason for the notable difference in reactivity between the isoflavone (14) and the pterocarpan (15) with citral during attempted introduction of the geranyl side chain is not apparent.

Although the presence of isoflavone-*C*-glycosides has been recorded in isolated genera of the Leguminoseae,[4-8] very few originate from *Dalbergia* species.[6-8] Continued examination of those present in the bark of *D. nitidula* [1] led to the isolation of four isoflavone-*C*-glycosides identified as 8-*C*-β-D-glucopyranosylgenistein (17a), 8-*C*-β-D-glucopyranosylorobol (17b), 6,8-di-*C*-β-D-glucopyranosylgenistein (17c, paniculatin [6]), and 6,8-di-*C*-β-D-glucopyranosylorobol (17d), accompanied by a 12a-hydroxy-rotenoid glycoside (5′*R*,6a*R*,12a*R*)-8′-*O*-β-D-glucopyranosyl-12a-hydroxyamorphigenin (18a). These glycosides occur in the bark in 0.86, 2.50, 0.97, 0.97, and 0.58% concentrations respectively, representing 4.0, 12.0, 4.5, 4.5, and 2.7% respectively of the methanolic extract. Concurrent work recorded in the Russian literature [9] indicates the isolation of the mono-glucosides (17a) and (17b) from *Lupinus luteus*. Similarly a substance, dalbin, of undefined stereochemistry and widely differing specific rotation, but obviously related to the rotenoid glycoside (18a), was isolated concurrently from the seeds of *Dalbergia latifolia*.[8] Our work includes the first assignment of their ^{13}C n.m.r. spectra and the absolute configuration of the rotenoid glycoside together with assessment of its relative toxicity.

None of the parent mono- and di-glycosides (17a—d)

undergoes either acidic or enzymic hydrolysis; one, 8-*C*-β-D-glucopyranosylorobol, was shown to furnish glucose on hydrolysis with iron(III) chloride,[10] but all readily yield the aglycones on treatment with hydro-

Assignments of resonances in the ^{13}C n.m.r. spectra of the aglycones genistein (17i) and orobol (17j) are based on allocations cited in the literature.[13] *C*-Glycosylation of these, as represented by the related

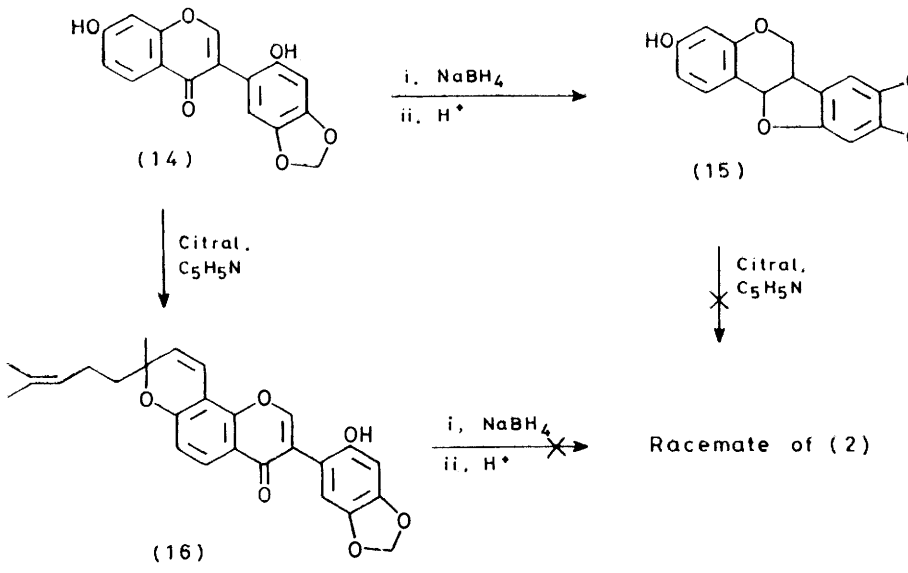


SCHEME 1 Complete synthesis of heminitidulan (1). Reagents: i, OH^-; ii, $Tl(NO_3)_3$; iii, H^+; iv, $MeOCH_2Cl$, OH^-, Adogen 464, CH_2Cl_2; v, H_2, Pd–C; vi, $PhCOCH_2Br$, K_2CO_3, Me_2CO; vii, citral, pyridine, 200 °C; viii, Zn, HOAc

iodic acid–phenol.[6] Thus, 4′,5,7-trihydroxyisoflavone (17i, genistein [11]) is obtained from both 8-*C*-β-D-glucopyranosylgenistein (17a) and the 6,8-diglucoside, paniculatin (17c), while 8-*C*-β-D-glucopyranosylorobol (17b) and 6,8-di-*C*-β-D-glucopyranosylorobol (17d) both afford 3′,4′,5,7-tetrahydroxyisoflavone (17j, orobol [12]).

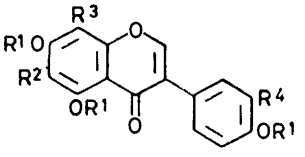
mono- (17a, 17b) and di-glycosides (17c, 17d) has no significant effect on the chemical shifts of the carbon resonances other than at the sites of glycosylation,[14] where an expected downfield shift of 8.5—10.2 p.p.m. relative to the signal of the corresponding aglycone is known to occur.[13] By comparison C-8 of the mono-

glycosides (17a) and (17b) exhibit deshielding of 8.5 and 9.8 p.p.m. respectively, while the same applies to both C-8 and C-6 of the diglycosides, paniculatin (17c) showing shifts of 8.8 (C-8) and 9.0 (C-6) p.p.m., and di-



SCHEME 2 Attempted synthesis of nitiducarpin

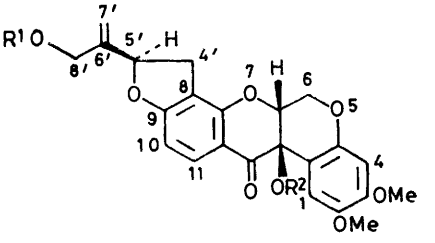
C-β-D-glucopyranosylorobol (17d) shifts of 9.7 and 9.3 p.p.m., respectively. Values for ^{13}C resonances associated with sugar units in the different compounds (17a—d) agree closely with each other and exhibit no significant deviation from those of known β-D-glucopyranosyl units attached to similar aglycones.[14] The foregoing spectrometric evidence defines both the sites of glycosylation and the identity of the sugar units, and hence confirms the structures for the isoflavone *C*-glycosides (17a—d).



(17)

a; $R^1=R^2=R^4=H$; $R^3=$β-D-Gl
b; $R^1=R^2=H$; $R^3=$β-D-Gl; $R^4=OH$
c; $R^1=R^4=H$; $R^2=R^3=$β-D-Gl
d; $R^1=H$; $R^2=R^3=$β-D-Gl; $R^4=OH$
e; $R^1=Ac$; $R^2=R^4=H$; $R^3=$β-D-GlAc
f; $R^1=Ac$; $R^2=H$; $R^3=$β-D-GlAc; $R^4=OAc$
g; $R^1=Ac$; $R^2=R^3=$β-D-GlAc; $R^4=H$
h; $R^1=Ac$; $R^2=R^3=$β-D-GlAc; $R^4=OAc$
i; $R^1=R^2=R^3=R^4=H$
j; $R^1=R^2=R^3=H$; $R^4=OH$
Gl = glucopyranosyl

A highly water-soluble 12a-hydroxyrotenoid glycoside (18a), $C_{29}H_{32}O_{13}$ (M^+ 588.181), similar to dalbin,[8] but differing substantially in the melting points of the amorphous compounds (125—127 and 161—163 °C, respectively) and their acetates (18b) (88—90 and 141—142 °C, respectively), and also in specific rotation ($[\alpha]_D$ —119.5 and —58.8° respectively) accompanies the above isoflavone *C*-glycosides. The ^1H n.m.r. spectrum of the acetate (18b) compares closely with that of dalbin penta-acetate,[8] small differences being ascribed to experimental error. Structural confirmation was obtained by acid hydrolysis to 6a,12a-didehydroamorphigenin [15] (19a), also characterized by its acetate

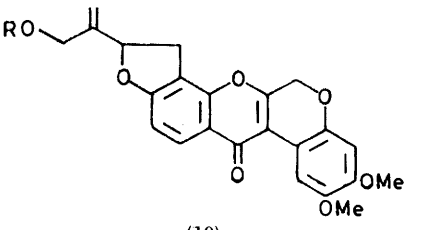


(18)

a; $R^1=$β-D-Gl; $R^2=H$
b; $R^1=$β-D-GlAc; $R^2=Ac$
c; $R^1=R^2=H$
d; $R^1=R^2=Ac$

(19b), and D-glucose. Enzymic hydrolysis (β-glucosidase) of the glycoside gives the aglycone (18c), $[\alpha]_D$ —122.5°, m.p. 102 °C, similar to dalbinol,[16] $[\alpha]_D$ —42.8°, m.p. 103—105 °C.

Comparative ^{13}C n.m.r. spectra of the glycoside (18a)



(19)

a; R=H
b; R=Ac

and its aglycone (18c) provide evidence of values for carbons associated with the sugar unit which are indistinguishable from those allocated to *O*-β-D-glucopyranosyl units.[17] The proposed [8] 8′-position of this glucose residue, leaving a free 12a-hydroxy-group in the parent glycoside, is confirmed by comparison of the ^{1}H n.m.r. spectra of the compound and its acetate (18a, 18b) with due reference to those of the aglycone and its acetate (18c, 18d). Acetylation results in deshielding of H-1 (δ 6.52 to 6.76; Δδ 0.24 p.p.m.) identical in magnitude and absolute value of the shift to that displayed by the aglycone with free 12a-hydroxy-functionality.

From its substantially higher melting points and low specific rotations it would appear that dalbin [8] is a partial racemate of 8′-*O*-β-D-glucopyranosyl-12a-hydroxyamorphigenin (18a), although the possibility of it being an epimeric 12a-hydroxy-isomer is not excluded.* Cotton effects in the c.d. spectra of the latter (18a) and its aglycone (18c) are almost identical to and show no significant deviation from those of (−)-*cis*-12a-hydroxyrotenone [18,19] {$[\alpha]_D$ ($CHCl_3$) −145°}, thus indicating a 5′*R*,6a*R*,12a*R* absolute configuration for the glycoside.

The presumed optical purity of the rotenoid glycoside prompted an assessment of its relative toxicity with the aglycone and also rotenone as references. Results show that its toxicity towards *Lucillia sericata* (Diptera) is low, comparable with or somewhat less than that of the aglycone.[20]

EXPERIMENTAL

Unless otherwise stated n.m.r. spectra were recorded for solutions in deuteriochloroform (Me_4Si as internal reference) and u.v. spectra in methanol. Mass spectra were obtained with a Varian CH-5 instrument, c.d. determinations with a JASCO J-20 spectropolarimeter, and specific rotation with a Bendix-NPL Automatic Polarimeter Type 143.

Systems used for separation of components comprised Whatman No. 3 paper (preparative paper chromatography) and Merck silica gel 60 PF_{254} (preparative t.l.c.). T.l.c. bands were located by u.v. illumination and perchloric acid-iron(III) chloride spray reagent.

In all cases, where omitted, mass spectral fragmentation and ^{1}H n.m.r. spectra are consistent with the proposed structures.

Synthesis of Heminitidulan [Racemate of (1)]

2,4-*Bis*(*methoxymethoxy*)*acetophenone* (3).—2,4-Dihydroxyacetophenone (2 g), sodium hydroxide (4 g), Adogen 464 (2 g), water (50 ml), and dichloromethane (50 ml) were stirred at room temperature (30 min).[21] Chloromethyl methyl ether (4 ml) was added dropwise and the mixture stirred for another hour. The organic layer was separated and the aqueous layer extracted with dichloromethane (3 × 50 ml). Preparative t.l.c. (benzene–acetone, 95 : 5) of the extracts yielded (3) as an *oil*, R_F 0.3, (2.2 g, 69%) (Found: M^+, 240.098. $C_{12}H_{16}O_5$ requires M, 240.100).

* *Note added in proof.* A sample of dalbinol kindly supplied by Dr. S. S. Chibber, Department of Chemistry, University of Delhi recently, exhibits the same c.d. spectrum as the aglycone (18c), indicating the identity also of dalbin from both sources in spite of recorded differences in m.p. and specific rotations.

2-*Benzyloxy*-4-*methoxybenzaldehyde* [22] (4).—A mixture of 2-hydroxy-4-methoxybenzaldehyde (5 g), benzyl chloride (4.5 ml), potassium carbonate (9 g), and dry dimethylformamide (DMF) (40 ml) was refluxed for 4 h (60 °C). After filtration, ice was added. Crystallization of the precipitate from methanol yielded needles of (4) (5.6 g, 71%), m.p. 69 °C (lit.,[22] 60 °C).

2-*Benzyloxy*-2′,4′-*bis*(*methoxymethoxy*)-4-*methoxychalcone* (5).—The acetophenone (3) (1.2 g) in ethanol (30 ml) and 50% (w/v) aqueous potassium hydroxide (10 ml) were stirred for 30 min at room temperature before addition of the aldehyde (4) (1.5 g). After 2 h water (100 ml) was added and the mixture extracted with diethyl ether (3 × 50 ml). The combined extracts were washed with water (2 × 50 ml) and taken to dryness. Purification of the product by t.l.c. (benzene–hexane–ethyl acetate, 5 : 4 : 1, R_F 0.23) gave the *chalcone* (5) (1.45 g, 65%) as a yellow oil (Found: M^+, 464.180. $C_{27}H_{28}O_7$ requires M, 464.183).

2′-*Benzyloxy*-7-*hydroxy*-4′-*methoxyisoflavone* (6).—Thallium(III) nitrate (1.12 g), the chalcone (5) (1.32 g), and methanol (150 ml) were stirred for 30 min, 3N hydrochloric acid (10 ml) was added, and the mixture refluxed for 5 h [23] and filtered hot. The product (6) crystallized as *needles* (800 mg, 75%), m.p. 226—228 °C (Found: C, 73.6; H, 5.0. $C_{23}H_{18}O_5$ requires C, 73.8; H, 4.9%), *m/e* 374 (41%, M^+).

2′-*Benzyloxy*-4′-*methoxy*-7-(*methoxymethoxy*)*isoflavone* (7).—2′-Benzyloxy-7-hydroxy-4′-methoxyisoflavone (6) (800 mg) was methoxymethylated as described for 2,4-dihydroxyacetophenone. Preparative t.l.c. (benzene–acetone, 95 : 5, R_F 0.35) and crystallization from ethanol yielded *needles* of (7) (550 mg, 61%), m.p. 134 °C (Found: C, 71.7; H, 5.3. $C_{25}H_{22}O_6$ requires C, 71.8; H, 5.3%), *m/e* 418 (62%, M^+); δ 8.16 (d, *J* 8.7 Hz, H-5), 7.85 (s, H-2), 7.25 (s, OCH_2Ph), 7.25 (d, *J* 8.7 Hz, H-6′), 7.03 (dd, *J* 8.7 and 2.5 Hz, H-6), 7.01 (d, *J* 2.5 Hz, H-8), 6.54 (d, *J* 2.5 Hz, H-3′), 6.53 (dd, *J* 8.7 and 2.5 Hz, H-5′), 5.21 (s, OCH_2-OMe), 5.03 (s, OCH_2Ph), 3.78 (s, 4′-OMe), and 3.50 (s, OCH_2OMe).

2′-*Hydroxy*-4′-*methoxy*-7-*methoxymethoxyisoflavan* (8).—The isoflavone (7) (500 mg) in ethanol (45 ml) and acetic acid (5 ml) was hydrogenated (room temp.; 3 atm; 6 h) with 10% palladium on charcoal (500 mg) as catalyst. Following filtration, water (50 ml) was added, the ethanol evaporated off, and the residue extracted with ether (3 × 50 ml). The extract was washed consecutively with 10% (w/v sodium hydrogencarbonate (2 × 50 ml) and water (2 × 50 ml), yielding the isoflavan on evaporation of the ether. Crystallization from ethanol gave *needles* of (8) (230 mg, 62%), m.p. 123 °C (Found: C, 68.0; H, 6.5. $C_{18}H_{20}O_5$ requires C, 68.3; H, 6.4%), *m/e* 316 (62%, M^+).

4′-*Methoxy*-7-*methoxymethoxy*-2′-*phenacyloxyisoflavan* (9).—A mixture of the preceding isoflavan (8) (234 mg), phenacyl bromide (148 mg), and potassium carbonate (500 mg) in dry acetone (20 ml) was refluxed for 2 h (60 °C).[24] The potassium carbonate was filtered off and the filtrate evaporated. Preparative t.l.c. (benzene–acetone, 98 : 2) gave the product (9) (R_F 0.38; 200 mg, 50%) which crystallized from ethanol as *needles*, m.p. 72 °C (Found: C, 71.8; H, 6.2. $C_{26}H_{26}O_6$ requires C, 71.9; H, 6.0%), *m/e* 434 (73%, M^+).

7-*Hydroxy*-4′-*methoxy*-2′-*phenacyloxyisoflavan* (10).—The isoflavan (9) (200 mg) was dissolved in methanol (5 ml) and 3N hydrochloric acid (1 ml) added dropwise. The mixture was refluxed for 1 h, and after addition of water

(20 ml) was extracted with ether (3 × 20 ml). Evaporation yielded the product as an *oil* (160 mg, 89%) (Found: M^+, 390.147. $C_{24}H_{22}O_5$ requires M, 390.147), *m/e* 390 (70%, M^+); δ 8.00—7.81 (m, H-2″,6″), 7.63—7.25 (m, H-3″,-4″,5″), 6.95 (d, *J* 7.5 Hz, H-6′), 6.85 (d, *J* 7.5, H-5), 6.54—6.21 (m, H-3′,5′,6,8), 5.23 (s, OCH_2COPh), 4.23 (dd, *J* 10 and 2.5 Hz, H-2$_{eq}$), 3.86 (d, *J* 10 Hz, H-2$_{ax}$), 3.71 (s, 4′-OMe), 3.81—3.44 (m, H-3), and 2.89 (d, *J* 7.5 Hz, H-4).

2′-O-*Phenacylheminitidulan* (12).—A mixture of the preceding isoflavan (10) (130 mg), citral (100 mg), and pyridine (0.5 ml) was heated in a fused glass tube for 12 h at 200 °C.[25] (The reaction was initially attempted at 150 °C, but no products were detected). After evaporation and preparative t.l.c. (benzene–ethyl acetate, 99 : 1) two components were obtained. 2′-O-*Phenacylheminitidulan* (12) (R_F 0.43; 20 mg, 11%) was isolated as an oil (Found: M^+, 524.254. $C_{34}H_{36}O_5$ requires M, 524.256); δ 7.97—7.78 (m, H-2,6 of CH_2COPh), 7.59—7.31 (m, H-3,4,5 of CH_2COPh), 6.97 (d, *J* 8 Hz, H-6′), 6.72 (d, *J* 8 Hz, H-5), 6.59 (d, *J* 10 Hz, H-4″), 6.41 (dd, *J* 8 and 2 Hz, H-5′), 6.29 (d, *J* 2 Hz, H-6′), 6.26 (d, *J* 8 Hz, H-6), 5.44 (d, *J* 10 Hz, H-3″), 5.22 (s, OCH_2COPh), 5.16—4.88 (m, H-7″), 4.34 (dd, *J* 10 and 2 Hz, H-2$_{eq}$), 4.00 (t, *J* 10 Hz, H-2$_{ax}$), 3.78—3.41 (m, H-3), 3.72 (s, 4′-OMe), 2.88 (d, *J* 10 Hz, H-4), 2.22—1.88 (m, H-6″), 1.81—1.50 (m, H-5″), 1.66 (s, 8″-Me), 1.59 (s, 8″-Me), and 1.37 and 1.39 (d, 2″-Me).

8-*Methyl*-8-(4-*methylpent*-3-*enyl*)-3-(2-*phenacyloxy*-4-*methoxyphenyl*)-3,4-*dihydro*-2H,8H-*benzo*[1,2-b;5,4-b′]-*dipyran* (11).—The second compound (R_F 0.34), isolated as an oil (20 mg, 11%) in the preceding reaction, was identified as the ' linear ' isomer (11) of 2′-*O*-phenacylheminitidulan (12) (Found: M^+, 524.256. $C_{34}H_{36}O_5$ requires M, 524.256); δ 7.94—7.75 (m, H-2,6 of CH_2COPh), 7.56—7.19 (m, H-3,4,5 of CH_2COPh), 6.95 (d, *J* 8 Hz, H-6′), 6.53 (s, H-5), 6.39 (dd, *J* 8 and 2 Hz, H-5′), 6.28 (d, *J* 2 Hz, H-3′), 6.19 (d, *J* 10 Hz, H-4″), 6.19 (s, H-8), 5.34 (d, *J* 10 Hz, H-3″), 5.19 (s, CH_2COPh), 5.19—4.88 (m, H-7″), 4.26 (dd, *J* 10 and 2 Hz, H-2$_{eq}$), 3.97 (t, *J* 10 Hz, H-2$_{ax}$), 3.69 (s, 4′-OMe), 3.75—3.44 (m, H-3), 2.84 (d, *J* 7.5 Hz, H-4), 2.19—1.88 (m, H-6″), 1.81—1.44, (m, H-5″), 1.62 (s, 8″-Me), 1.56 (s, 8″-Me), and 1.34 (s, 2″-Me).

(±)-*Heminitidulan* [*racemate of* (1) [4]].—Zinc powder (100 mg) was added portionwise to a stirred solution of 2′-*O*-phenacylheminitidulan (12) (15 mg) in acetic acid (2 ml); after the mixture had been heated for 1 h on a steam bath, water (20 ml) was added and the solution extracted with ether (3 × 20 ml). The combined ether extracts were washed with 10% (w/v) sodium hydrogen carbonate (20 ml). Evaporation produced the racemic heminitidulan (10 mg), which apart from a narrowly split 2″-Me resonance was otherwise (m.s. and ^{1}H n.m.r.) identical with the optically active natural product,[1] and was obtained as an *amorphous solid*, m.p. 80 °C (Found: M^+, 406.215. $C_{26}H_{30}O_4$ requires M, 406.214); δ 1.27, 1.29 (d, 2″-Me).

Attempts at Synthesis of Nitiducarpin

3-(2-*Hydroxy*-4,5-*methylenedioxyphenyl*)-8-*methyl*-8-(4-*methylpent*-3-*enyl*)-4H,8H-*benzo*[1,2-b;3,4-b′]*dipyran*-4-*one* (16).—A solution of 2′,7-dihydroxy-4′,5′-methylenedioxyisoflavone [23] (14) (260 mg) and citral (260 mg) in 0.5 ml of pyridine was heated at 200 °C for 12 h. Separation of the mixture by t.l.c. (benzene–ethyl acetate, 99 : 1) gave (16) as the main product as an *oil* (50 mg, 13%; R_F 0.77) (Found: M^+, 432.160. $C_{26}H_{24}O_6$ requires M, 432.157); δ 9.72 (s, 2′-OH), 7.84 (s, H-2), 7.52 (d, *J* 7.5 Hz, H-5), 7.25 (s, H-6′), 6.90 (s, H-3′), 6.70 (d, *J* 10 Hz, H-4″), 6.24 (d, *J* 7.5 Hz, H-6), 5.91 (s, OCH_2O), 5.47 (d, *J* 10 Hz, H-3″), 5.19—4.88 (br t, H-7″), 2.31—1.87 (m, H-6″), 1.87—1.56 (m, H-5″), 1.62 (s, 8″-Me), 1.56 (s, 8″-Me), and 1.40 (s, 2″-Me).

Attempted Reduction of the Pyranoisoflavone (16).—Reduction of the pyranoisoflavone (16) with sodium borohydride, under those conditions, which permitted satisfactory conversion of 2′,7-dihydroxy-4′,5′-methylenedioxyflavone (14) into the pterocarpan analogue (15), gave complex mixtures of products from which the desired nitiducarpin [racemate of (2)] could not be isolated.

Attempted Introduction of a Geranyl Side-chain into 3-*Hydroxy*-8,9-*methylenedioxypterocarpin* (15).—The synthetic pterocarpin (15) (300 mg), obtained by reduction ($NaBH_4$) [2] of 2′,7-dihydroxy-4′,5′-methylenedioxyisoflavone (14) was treated with citral (300 mg) in 0.5 ml of pyridine at 200 °C for >12 h. Only starting materials were recovered, with no chromatographic evidence of product formation.

Isolation and Identification of Glycosides from Dalbergia nitidula *Bark*

The bark (pulverised and dried; 718 g) was extracted with methanol (6 × 1 l) at room temperature for 6 consecutive days and gave a brown solid (155 g) on evaporation of the combined extracts. Preparative paper chromatography (2% AcOH; upward migration) of a portion (30 g) of the extract yielded three crude fractions (A, R_F 0.01, 5.2 g; B, 0.41, 6 g; C, 0.78, 5.5 g). Fraction A has been investigated previously.

Separation of fraction B (1 g) by t.l.c. ($CHCl_3$–Me_2CO–MeOH–H_2O, 44 : 44 : 2 : 10) gave two components (17a) and (17b).

8-C-β-D-*Glucopyranosylgenistein* (17a).—The substance with the higher R_F value (0.55) proved to be 8-*C*-β-D-glucopyranosylgenistein (17a) (200 mg, light yellow amorphous solid), m.p. >350 °C; ^{13}C n.m.r. δ(CD_3SOCD_3) 175.9 (C-4), 156.7 (C-7), 156.7 (C-4′), 154.2 (C-8a), 154.2 (C-5), 149.2 (C-2), 127.5 (C-2′), 127.5 (C-6′), 120.0 (C-1′), 118.8 (C-3), 112.8 (C-3′), 112.8 (C-5′), 102.2 (C-8), 100.1 (C-4a), 98.6 (C-6), 79.0 (C-5″), 77.7 (C-1″), 72.7 (C-2″), 69.0 (C-3″), 68.4 (C-4″), and 59.0 (C-6″) p.p.m.

Hepta-O-*acetyl*-8-C-β-D-*glucopyranosylgenistein* (17e).—Acetylation of 8-*C*-β-D-glucopyranosylgenistein (17a) (20 mg) with acetic anhydride–pyridine gave (17e) as a light yellow amorphous solid, m.p. 108—110 °C.

Genistein (17i).—A mixture of 8-*C*-β-D-glucopyranosylgenistein (17a) (100 mg), phenol (600 mg), and hydroiodic acid (1 ml, *d* 1.7) was refluxed (135 °C) for 7 h. The cooled mixture was poured into stirred 20% aqueous sodium hydrogensulphite. A brown substance settled which was separated by t.l.c. ($CHCl_3$–Me_2CO, 8 : 2) to yield genistein (17i) (R_F 0.44, 25 mg), m.p. 300 °C (lit.,[11,26] 300—301 °C); *m/e* 270 (100%, M^+); ^{13}C n.m.r. δ(CD_3SOCD_3) 180.2 (C-4), 164.0 (C-7), 162.2 (C-4′), 157.6 (C-8a), 157.6 (C-5), 153.8 (C-2), 130.0 (C-2′), 130.0 (C-6′), 122.6 (C-1′), 121.3 (C-3), 115.0 (C-3′), 115.0 (C-5′), 104.2 (C-4a), 98.6 (C-6), and 93.9 (C-8) p.p.m.

8-C-β-D-*Glucopyranosylorobol* (17b).—The second component of fraction B (R_F 0.42; 600 mg), identified as 8-*C*-β-D-glucopyranosylorobol (17b), was isolated as a light yellow amorphous solid, m.p. 265 °C; ^{13}C n.m.r. δ(CD_3SOCD_3) 179.1 (C-4), 163.2 (C-7), 159.8 (C-8a), 154.8 (C-5), 152.4 (C-2), 144.6 (C-3′), 144.0 (C-4′), 121.6 (C-3), 121.0

(C-1′), 119.1 (C-6′), 116.0 (C-2′), 114.7 (C-5′), 103.8 (C-8), 103.5 (C-4a), 98.8 (C-6), 80.8 (C-5″), 78.4 (C-1″), 73.1 (C-2″), 70.3 (C-3″), 70.3 (C-4″), and 61.3 (C-6″) p.p.m.

8-*C*-β-D-Glucopyranosylorobol (17b) (100 mg) was hydrolysed with iron(III) chloride (500 mg) in water (1.6 ml) under reflux for 6 h and the product worked up as described in the literature.[10] Examination by paper chromatography (EtOAc–pyridine–water, 10 : 4 : 3, v/v) led to the detection of glucose using *p*-anisidine hydrochloride spray reagent.[27]

Hepta-O-acetyl-8-C-β-D-glucopyranosylorobol (17f).—The product (20 mg) was obtained by acetylation of 8-*C*-β-D-glucopyranosylorobol (17b) (25 mg) as an amorphous solid, m.p. 120 °C; *m/e* 784 (5%, M^+).

Orobo l(17j).—Treatment of 8′-*C*-β-D-glucopyranosylorobol (17b) (100 mg) with phenol (600 mg) and hydroiodic acid (1 ml) (as previously described) yielded orobol (17j) (20 mg) as an amorphous solid, m.p. 270 °C (lit.,[12] 270.5 °C); *m/e* 286 (16%, M^+); ^{13}C n.m.r. δ(CD_3SOCD_3) 177.9 (C-4), 162.3 (C-7), 159.8 (C-8a), 155.5 (C-5), 151.7 (C-2), 143.6 (C-3′), 143.0 (C-4′), 120.6 (C-3), 120.0 (C-1′), 118.2 (C-6′), 115.0 (C-2′), 113.8 (C-5′), 102.6 (C-4a), 97.6 (C-6), and 92.3 (C-8) p.p.m.

Column chromatography ($CHCl_3$–EtOH, 8 : 2) of fraction C (2 g) yielded two subfractions (C_1 and C_2).

(−)-8′-C-β-D-*Glucopyranosyl*-12a-*hydroxyamorphigenin* (18a).—Fraction C_1 (R_F 0.35; 300 mg) produced a single compound (identified as an optically pure form of dalbin[8]), which was isolated as an amorphous solid, m.p. 125—127 °C (lit.,[8] 161—163 °C); $[\alpha]_D$ −119.5° (*c* 0.45 in MeOH) (lit.,[8] $[\alpha]_D$ −58.8°) (Found: M^+, 588.181. $C_{29}H_{32}O_{13}$ requires *M*, 588.184), *m/e* 588 (10%, M^+), 426 (6), 219 (21), 208 (100), 207 (69), 193 (11), 191 (7), and 165 (14); $\lambda_{max.}$ (log ε) 292 (4.45), 240 (4.47), and 205 (4.72) nm; c.d. (*c* 0.044 4) $[\theta]_{350}$ 0, $[\theta]_{320}$ −13 240, $[\theta]_{295}$ −4 200, $[\theta]_{275}$ −13 300, $[\theta]_{253}$ 0, $[\theta]_{239}$ 18 000, $[\theta]_{230}$ 0; δ[$(CD_3)_2CO$ + D_2O] 7.65 (d, *J* 8.7 Hz, H-11), 6.52 (s, H-1), 6.45 (d, *J* 8.7 Hz, H-10), 6.38 (s, H-4), 5.42 (t, *J* 8.7 Hz, H-5′), 5.22 (d, *J* 5 Hz, H-7′), 4.66 (s, Gl-H), 4.56—4.44 (m, H-6, 6a), 4.38—3.88 (m, H-8′, Gl-H), 3.69 (s, OMe), 3.56 (s, OMe), and 3.44—2.81 (m, H-4′, 5 × Gl-H); ^{13}C n.m.r. δ(C_5D_5N) 190.6 (C-12), 166.0 (C-9), 156.7 (C-7a), 150.8 (C-3), 148.0 (C-4a), 144.0 (C-6′), 143.0 (C-2), 129.1 (C-11), 112.7 (C-11a), 112.6 (C-8), 111.3 (C-1), 109.5 (C-7′), 109.5 (C-12b), 105.0 (C-10), 104.9 (C-1″), 101.0 (C-4), 84.3 (C-5′), 78.7 (C-3″), 77.5 (C-5″), 76.2 (C-6a), 74.1 (C-2″), 70.6 (C-4″), 68.1 (C-8′), 67.9 (C-6), 61.9 (C-6″), 61.9 (C-12a), 55.7 (OMe), 55.1 (OMe), and 31.0 (C-4′) p.p.m.

2″,3″,4″,6″,12a-*Penta*-O-*acetyl*-8′-C-β-D-*glucopyranosylamorphigenin* (18b).—Acetylation of the glycoside (18a) (20 mg) gave the penta-acetate (18b) (18 mg) as an amorphous solid, m.p. 88—90 °C (lit.,[8] 141—142 °C), *m/e* 738 (96%; M^+ − AcOH).

6a,12a-*Didehydroamorphigenin* (19a).—The rotenoid glycoside (18a) (100 mg) was refluxed for 1 h with 3N hydrochloric acid (5 ml). After addition of water (10 ml), the mixture was extracted with ether (3 × 30 ml). Evaporation of the ether extract and crystallization from methanol yielded 6a,12a-didehydroamorphigenin (19a) (50 mg) as needles, m.p. 233 °C (lit.,[15] 228.5—229.5 °C); *m/e* 408 (100%; M^+); c.d. (*c* 0.044 4) $[\theta]_{350}$ 0, $[\theta]_{320}$ −1 300, $[\theta]_{295}$ −2 700, $[\theta]_{275}$ −4 600, $[\theta]_{253}$ 0; δ(C_5D_5N) 8.90 (s, H-1), 8.40 (d, *J* 8.0 Hz, H-11), 7.05 (d, *J* 8.0 Hz, H-10), 6.83 (s, H-4), 5.92 (d, *J* 8.0 Hz, H-5′), 5.58 (d, *J* 8.0 Hz, H-7′), 5.13 (s, H-6), 4.65 (s, H-8′), 3.83 and 3.80 (s, 2 × OMe), and 3.53—3.37 (m, H-4′).

Glucose.—Evaporation of the aqueous layer in the preceding hydrolysis yielded a substance which showed the same R_F value (0.1, EtOAc–pyridine–H_2O, 12 : 5 : 4) by paper chromatography and colour reaction (yellow) with *p*-anisidine hydrochloride spray reagent[27] as commercial D-glucose.

8′-O-*Acetyl*-6a,12a-*didehydroamorphigenin* (19b).—6a,12a-Didehydroamorphigenin (19a) (20 mg) was acetylated to yield the monoacetate (19b) (18 mg) as yellow needles (from MeOH), m.p. 182 °C (lit.,[15] 179 °C); *m/e* 450 (100%, M^+).

(−)-12a-*Hydroxyamorphigenin* (18c).—The rotenoid glycoside (18a); (200 mg) and β-glucosidase (200 mg) were suspended in a sodium acetate buffer solution (pH 5.0). The mixture was incubated for 24 h at 36 °C. Extraction with ether (3 × 50 ml), evaporation and t.l.c. separation (benzene–acetone, 8 : 2) gave the aglycone (18c) (R_F 0.29; 120 mg) as an amorphous solid, m.p. 102 °C (lit.,[16] 103—105 °C); $[\alpha]_D$ −122.5° (*c* 1 in MeOH) (lit.,[16] −42.8°); *m/e* 426 (64%; M^+); c.d. (*c* 0.044 4) $[\theta]_{350}$ 0, $[\theta]_{320}$ −15 800 $[\theta]_{295}$ −4 300, $[\theta]_{275}$ −16 800, $[\theta]_{253}$ 0, $[\theta]_{237}$ 26 000, $[\theta]_{230}$ 0; ^{13}C n.m.r. δ(C_5D_5N) 190.3 (C-12), 166.6 (C-9), 156.1 (C-7a), 150.5 (C-3), 148.0 (C-4a), 148.0 (C-6′), 143.0 (C-2), 128.8 (C-11), 112.6 (C-8), 112.6 (C-11a), 111.3 (C-1), 108.8 (C-7′), 108.8 (C-12b), 103.9 (C-10), 100.7 (C-4), 84.6 (C-5′), 75.9 (C-6a), 67.8 (C-6), 63.4 (C-8′), 60.9 (C-12a), 55.4, and 54.7 (2 × OMe), and 31.1 (C-4′) p.p.m.

8′-O-*Acetyl*-12a-*acetoxyamorphigenin* (18d).—The product (18d) (18 mg) obtained by acetylation of (−)-12a-hydroxyamorphigenin (18c) is an amorphous solid, m.p. 76 °C (lit.,[16] 80 °C); *m/e* 510 (5.4%; M^+).

Fraction C_2 (1.2 g) was re-chromatographed (t.l.c.; $CHCl_3$–Me_2CO–MeOH–H_2O, 35 : 35 : 10 : 20, 2×) into two components (17c) and (17d).

Paniculatin (17c).—The substance with R_F 0.37 (500 mg) crystallized from water as needles, m.p. 225 °C (lit.,[6] 225—227 °C); ^{13}C n.m.r. δ(CD_3SOCD_3) 175.9 (C-4), 157.8 (C-7), 154.7 (C-4′), 154.7 (C-8a), 153.5 (C-5), 149.1 (C-2), 127.5 (C-2′), 127.5 (C-6′), 120.0 (C-1′), 119.4 (C-3), 113.1 (C-3′), 113.1 (C-5′), 107.6 (C-6), 102.2 (C-4a), 102.4 (C-8), 81.5 (C-5″), 79.6 (C-5‴), 77.7 (C-1″), 75.8 (C-1‴), 73.4 (C-2″), 71.5 (C-2‴), 70.4 (C-3″), 69.0 (C-3‴), 69.0 (C-4″), 69.0 (C-4‴), 60.9 (C-6″), and 59.7 (C-6‴) p.p.m.

Paniculatin Acetate (17g).—Acetylation of paniculatin (17c) (20 mg) gave the acetate (17g) (22 mg) as an amorphous solid, m.p. 160 °C (lit.,[6] 161—162 °C).

Paniculatin (17c) (100 mg), when refluxed with hydroiodic acid (1 ml) and phenol (600 mg) gave genistein[9,18] (20 mg) which was identical with that obtained from 8-β-D-glucopyranosylgenistein (17a).

6,8-*Di*-C-β-D-*glucopyranosylorobol* (17d).—The second compound (17d) (R_F 0.28; 500 mg) from fraction C_2 was isolated as a *yellow amorphous solid*, m.p. 183—186 °C; ^{13}C n.m.r. δ(CD_3SOCD_3) 177.5 (C-4), 162.3 (C-7), 157.3 (C-8a), 152.9 (C-5), 150.5 (C-2), 143.0 (C-3′), 142.4 (C-4′), 120.0 (C-3), 120.0 (C-1′), 118.1 (C-6′), 114.7 (C-2′), 113.8 (C-5′), 106.9 (C-6), 102.3 (C-4a), 102.0 (C-8), 80.8 (C-5″), 79.6 (C-5‴), 77.1 (C-1″), 75.8 (C-1‴), 73.0 (C-2″), 71.5 (C-2‴), 70.3 (C-3″), 68.4 (C-3‴), 68.4 (C-4″), 68.4 (C-4‴), 60.9 (C-6″), and 59.3 (C-6‴) p.p.m.

O-*Acetyl*-6,8-*di*-C-β-D-*glucopyranosylorobol* (17h).—The acetate (17h) (22 mg) was obtained by acetylation of 6,8-di-*C*-β-D-glucopyranosylorobol (17d) (20 mg) as an *amorphous solid*, m.p. 150 °C; δ 6.07 (s, H-2), 7.50—7.27 (m, H-2′,5′,6′), 5.60—5.00 (m, H-1″,2″,3″,4″,1‴,2‴,3‴,4‴), 4.50—3.50 (m, H-5″,6″,5‴,6‴), 2.53 (s, 5-OAc), 2.47 (s,

7-OAc), 2.30 (s, 3′,4′-OAc), 2.07 (s, 3″,4″,6″,3‴,4‴,6‴-OAc), 1.88 (s, 2″-OAc), and 1.73 (s, 2‴-OAc).

Treatment of 6,8-di-*C*-β-D-glucopyranosylorobol (100 mg) with hydroiodic acid (1 ml) and phenol (600 mg) produced orobol [12] (25 mg) similar to that obtained from 8-β-D-glucopyranosylorobol.

Toxicity Determinations on Rotenoids

The reaction rate for enzyme (ferricytochrome c reductase) catalysed reduction of ferricytochrome c by NADH was determined by following the disappearance of the former at 550 nm with a Durrum Model D-110 stopped-flow spectrophotometer. Attempts at determining the relative toxicity of the rotenoid glycoside (18a) and its aglycone (18c) compared with that of rotenone by studying the decrease in the reaction rate (enzymic inhibition) on addition of small amounts of these compounds gave variable results.

LD_{50} determinations were accordingly resorted to. The Table shows the mortality of groups of twenty *Lucillia sericata* (Diptera) over a 24 h period when injected with the rotenoid compounds dissolved in dimethyl sulphoxide. Two controls give mortalities of 0 and 1.

	Dose/μg						
Compound	4	3.5	3.0	2.5	2.0	1.75	1.0
Rotenone	19	18	17	13	11	9	9
12a-Hydroxyamorphigenin (18a)	6	0	4	2	2	0	4
Glycoside of 12a-hydroxyamorphigenin (18c)	1	1	3	0	1	4	4

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