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GLAUCIN B, A NEW BITTER LIMONOID FROM *EVODIA GLAUCA*

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Key Word Index—*Evodia glauca*; Rutaceae; bitter limonoid, limonin, rutaevin, limonin diosphenol, glaucin A, 5β-H limonoid

Abstract—A new bitter limonoid, glaucin B, has been isolated from the root bark of *Evodia glauca* together with the known limonoids limonin, rutaevin, limonin diosphenol and glaucin A (12α-hydroxyrutaevin). The structure of glaucin B determined to have the uncommon 5β-H configuration by spectral and chemical evidence.

INTRODUCTION

In the course of our studies on the biologically active limonoids from Meliaceae and Rutaceae plants [1–5], we have isolated a new limonoid glaucin B along with the known limonoids, limonin, rutaevin, limonin diosphenol and glaucin A (12α-hydroxyrutaevin), as bitter components of the root bark of *Evodia glauca* Miq., which is a large tree found in the southwest islands of Japan and resembles the Japanese Meliaceae *Melia azedarach* L. var. Japonica Makino. The structure and stereochemical assignments of glaucin B were made using COSY and NOESY spectroscopy, which indicated the uncommon 5β-H configuration of the new compound which is opposite to that of many known Rutaceae limonoids including limonin. The structure was related chemically to the known limonoids. Some biological activities of the limonoids isolated in this work were also examined.

RESULTS AND DISCUSSION

The ether extract of the root bark (1 kg) contained many limonoids which were detected by the characteristic colour with Ehrlich's reagent on TLC. Fractionation by extensive silica gel column chromatography followed by the final HPLC purification on a normal phase column afforded a new limonoid glaucin B (**1**) along with limonin [6], rutaevin [7], limonin diosphenol (**3**) [8, 9] and glaucin A [5].

Glaucin B (**1**), mp 228–231° as powder from MeOH, $[\alpha]_D^{15}$ +29° (MeOH, *c* 0.001), was assigned the molecular formula $C_{28}H_{32}O_{10}$ from FDMS (*m/z* 529 $[M+H]^+$). Its UV, IR and CD spectra showed the presence of furan, δ- and γ-lactone, ester and ketone groups. The ^{1}H NMR spectrum of **1** showed signals of four tertiary methyl groups at δ 1.26 (8-Me), 1.28 (4β-Me), 1.32 (13-Me) and 1.37 (4α-Me), one acetyl singlet at 2.21, a characteristic sharp epoxide proton singlet at 3.95 (H-15), an AB quartet (J = 15 Hz) at 4.07 (H-19a) and 4.31 (H-19b), and a singlet at 5.61 (H-17), along with the usual limonoid furan signals at 7.44 (*dd*, J = 1.5 and 1 Hz; H-21), 6.31 (*dd*, J = 1.5 and 1 Hz; H-22) and 7.42 (*t*, J = 1.5 Hz; H-23). The H-17 signal showed small allylic couplings with the H-21 and H-22 signals [2–5] and the H-19a signal showed a *W*-type long-range coupling with a signal at δ 4.25 (1H, *br t*, J = 3 Hz; H-1), which revealed the H-1 to be in the α-configuration [5]. Furthermore, the ^{1} NMR spectrum showed an acetoxy methine proton at δ 5.54 (*d*, J = 11 Hz, H-6) coupled with a doublet methine proton at δ 2.34 (*d*, J = 11 Hz; H-5). 2D-Homonuclear J-correlation (COSY) spectrum clarified the coupling protons on the linked carbons (C-1 and C-2, C-5 and C-6, and C-9, C-11 and C-12). These ^{1}H NMR spectral features resembled those of limonin except for the presence of the signals due to an additional acetoxyl group (see Experimental).

In the ^{1}H NOE experiments on **1**, irradiation of the 8-Me protons at δ 1.26 induced 6.9, 4.1, 2.2 and 1.3% peak enhancements on the H-15α, H-19a, H-19b and H-5 signals, respectively. The NOE on the H-5 suggested the β-configuration which is opposite to that of limonin, in the spectrum of which irradiation of the 8-Me protons at δ 1.07 showed the following NOEs: 5.5(H-6β), 13.5 (H-15), 2.5 (H-19a) and 6.7% (H-19b). The 5β-H configuration was also supported by 10% peak enhancement on the H-5 signal by irradiation of the 4β-Me protons at δ 1.28. On the other hand, irradiation of the 4α-Me protons at δ 1.37 induced 4.9 and 10.2% peak enhancements on the H-1α and H-6 signals, respectively, which clarified the β-configuration of the 6-acetoxyl group. Therefore, compound **1** should be the 6β-acetoxy-5β-H analogue of limonin, which has the same A/B *cis*-ring junction with that of rutaevin and glaucin A. Irradiation of the 4α-Me (δ 1.21) and 4β-Me (δ 1.44) protons in the spectrum of rutaevin induced 9.5 and 12.0% NOEs on the H-1α and H-5 signals, respectively. Detailed NOE data on rutaevin and graucin A are given in the Experimental section. The ^{1}H NMR spectrum of **1** (Table 1) supports the structure and the cross-relaxation correlated 2D-^{1}H NOE (NOESY) (4α-Me/H-5, H-15, H-19a, H-19b and 13-Me/H-9, H-21, H-22) revealed the gross conformation (Fig. 1).

Hydrolysis of **1** with 0.1 N aq. sodium carbonate in MeOH followed by Jones oxidation, afforded an α-dike-

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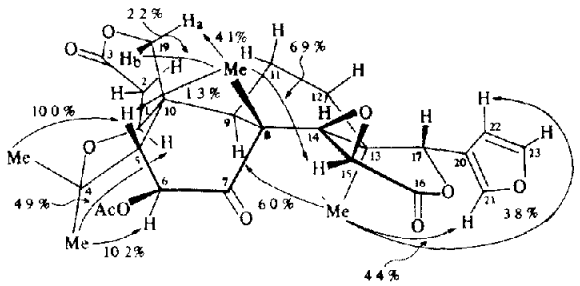


Fig 1 Glaucin B, stereochemistry and NOEs between methyl and proton

tone **2**, mp 283–286°, CD(MeOH) $\Delta\varepsilon_{407}-1.0$ (n-π^* of diketo), which was also derived from rutaevin by Jones oxidation (Scheme 1) Thus compound **1** was demonstrated to have the same stereochemistry as rutaevin These reactions and the ^{1}H NMR data led to the assignment of the structure of glaucin B as **1**

When left in methanol for 1 day, the dione **2** was partly converted into a diosphenol **3** (limonin diosphenol) to give a mixture as shown from spectral data [1], λ_{max}^{MeOH} 208 (π–π^* of furan), 277 nm (π–π^* of **3**); CD (MeOH) $\Delta\varepsilon_{211}$ (π–π^* of furan), $\Delta\varepsilon_{272}$ (π–π^* of **3**), $\Delta\varepsilon_{319}$ (n–π^* of **2**) (Fig 2) On the other hand, oxidation of the hydrolysis product from **1** with pyridinium chlorochromate afforded the diosphenol **3**, which was also obtained from the dione **2** by treatment with alkali. The formation of compound **3** has been reported from limonin and rutaevin [7, 8].

Although there have been many Rutaceous limonoids reported, the 5β-H compounds are rare [5, 10]. Among the compounds isolated in this work, limonin and limonin diosphenol (**3**) were active as an insect antifeedant against the larvae of *Spodoptera litura* Fab., but glaucin B (**1**) showed no activity On the other hand, in antimicrobial tests against bacteria, yeasts and fungi rutaevin showed weak antibacterial activity against *Bacillus subtilis*

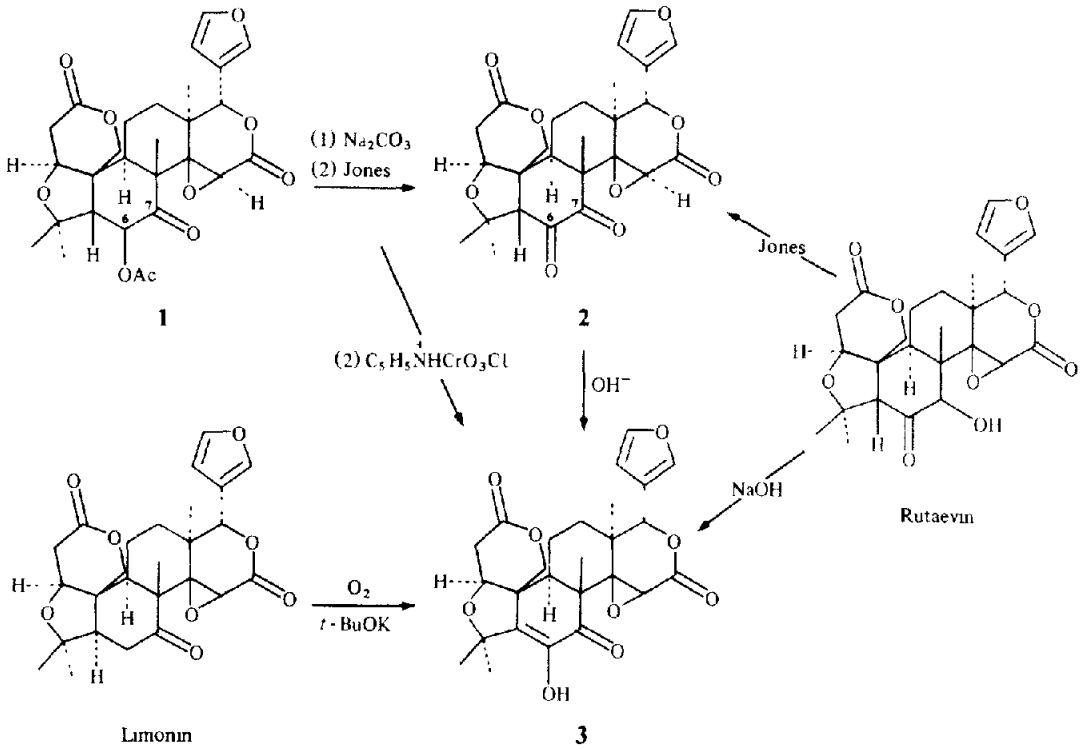
EXPERIMENTAL

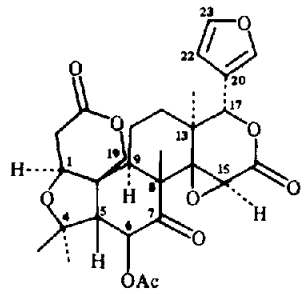
Mps uncorr UV spectra were measured in MeOH and ^{1}H NMR spectra in $CDCl_3$ with TMS as int standard were measured at 360 MHz

Plant material Roots of the plant were collected at the botanical garden of Kagoshima University in August 1983 and identified by Dr S Sako (Kagoshima University)

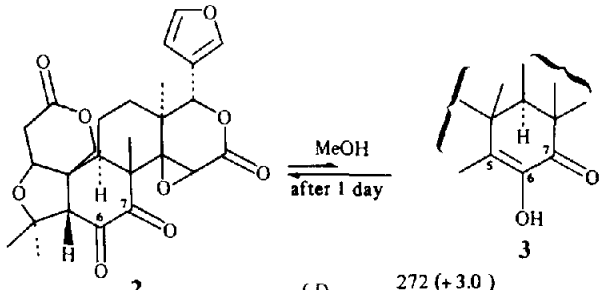
Extraction and isolation Fresh root bark (1.0 kg) was extracted for 1 week with MeOH (3 × 3l) at room temp After concn to 200 ml, H_2O (300 ml) was added to afford 5.2 g of a ppt which was applied onto a silica gel column The column was eluted with a CH_2Cl_2–MeOH gradient to yield a complex limonoid mixture and glaucin A (13 mg) The mixture was separated by repeated passage through a HPLC, semiprep silica gel column, using 0.4–1.6% MeOH–CH_2Cl_2 as the solvent to give **1** (26 mg), limonin (320 mg), rutaevin (26 mg) and limonin diosphenol (**3**, 21 mg)

Glaucin B (**1**) Powder from MeOH, mp 228–231°, $[\alpha]_D^{15}+29°$ (MeOH, c 0.001), UV λ_{max}^{MeOH} nm (log ε) 209 (3.72), 279 (2.43), IR ν_{max}^{Nujol} cm^{-1} 1760 (γ-lactone), 1735 (δ-lactone and ester), 1700 (ketone), CD (MeOH) nm $\Delta\varepsilon_{213}$ −4.0 (π–π^* of furan), $\Delta\varepsilon_{234}$ −4.7 (n–π^* of lactone and ester), $\Delta\varepsilon_{296}$ +5.9, $\Delta\varepsilon_{305}$ +5.6, $\Delta\varepsilon_{315sh}$ +2.3 (n–π^* of ketone), FDMS m/z 529 $[M-1]^+$, 469, 431, 265, 183, 129, ^{1}H NMR see Table 1





1



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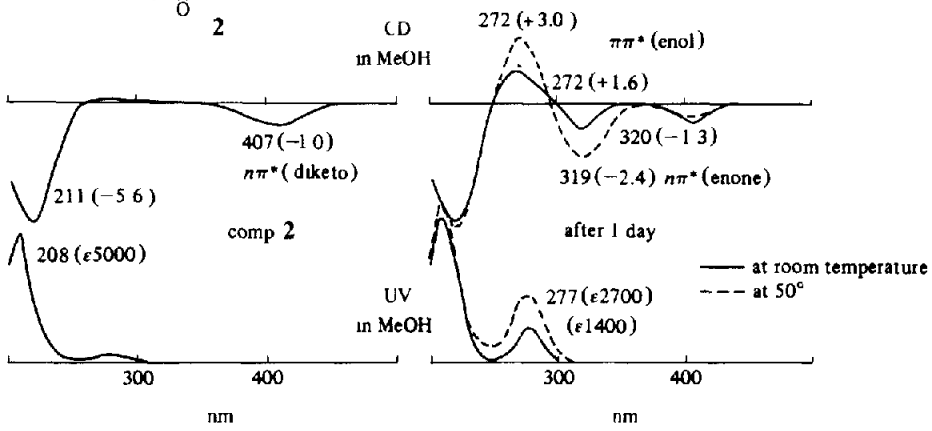

Fig. 2

Table 1 ^{1}H NMR data of Glaucin B (1) ($CDCl_3$, 360 MHz)

H	δ	Mult	J (Hz)	Coupled to	H	δ	Mult	J (Hz)	Coupled to
1	4.25	*brt*	3	2a, 2b, (19a)*	17	5.61	*brs*		(21), (22)
2a	2.64	*dd*	3, 16	1, 2b	18	1.32	*s*		
2b	2.89	*dd*	3, 16	1, 2a	19a	4.07	*brd*	15	19b, (1)
5	2.34	*d*	11	6	19b	4.31	*d*	15	19a
6	5.54	*d*	11	5	21	7.44	*brdd*	1, 1.5	22, 23, (17)
9	3.19	*dd*	5, 13	11α, 11β	22	6.31	*brdd*	1, 1.5	21, 23, (17)
11α	1.79	*m*		9, 11β, 12α, 12β	23	7.42	*t*	1.5	21, 22
11β	1.93	*m*		9, 11α, 12α, 12β	28	1.37	*s*		
12α	1.63	*m*		11α, 11β, 12β	29	1.28	*s*		
12β	1.82	*m*		11α, 11β, 12α	30	1.26	*s*		
15	3.95	*s*			OAc	2.21	*s*		

*Distinguished couplings are listed and numerals in parenthesis denote small coupling based on COSY.

Limonin. Prisms from MeOH; mp 289–293°; $[\alpha]_D^{24}-136°$ (Me_2CO, *c* 0.004); UV λ_{max}^{MeOH} nm(log ε). 208(3.86); IR ν_{max}^{Nujol} cm^{-1} 1760, 1705, 1503, 878; CD(MeOH) nm $\Delta\varepsilon_{230}-4.9$, $\Delta\varepsilon_{293}-2.3$, EIMS *m/z*: 470 $[M]^+$, 455, 426, 413, 347, 135, 108, 94, ^{1}H NMR: δ 1.07 (3H, *s*, 8-Me), 1.18 (3H, *s*, 4β-Me), 1.18 (3H, *s*, 13-Me), 1.30 (3H, *s*, 4α-Me), 1.52 (1H, *m*, H-12α), 1.78 (1H, *m*, H-12β), 1.83 (1H, *m*, H-11α), 1.90 (1H, *m*, H-11β), 2.23 (1H, *dd*, *J* = 16 and 3 Hz, H-5), 2.47 (1H, *dd*, *J* = 14.5 and 3.5 Hz, H-6α), 2.55 (1H, *dd*, *J* = 12

and 2 5 Hz, H-9), 2 68 (1H, *dd*, $J=17$ and 2 Hz, Ha-2), 2 86 (1H, *dd*, $J=15$ 5 and 14 5 Hz, H-6β), 2 98 (1H, *dd*, $J=17$ and 4 Hz, H_b-2), 4 03 (1H, *m*, H-1), 4 03 (1H, *s*, H-15), 4 47 (1H, *d*, $J=13$, H_a-19), 4 77 (1H, *br d*, $J=13$, H_b-19), 5 47 (1H, *br s*, H-17), 6 34 (1H, *ddd*, $J=2$, 1 and 1 Hz, H-22), 7 40 (1H, *dd*, $J=2$ and 1 5 Hz, H-23), 7 42 (1H, *dd*, $J=1$ 5 and 1 Hz, H-21) NOEs (no indication in parentheses based on 2D-NOE) H-9/H-1, H-5, 13-Me, 13-Me/H-9 (5 8%), H-21 (4 3%), H-22 (5%), 4α-Me/H-5 (6 9%), H-1 (2 5%), 4β-Me/H-19b, 8-Me/H-6β (5 5%), H-15 (13 5%), H_a-19 (2 5%), H_b-19 (6 7%)

Rutaevin Needles from MeOH, mp > 300°, $[\alpha]_D^{24}$ −146° (MeOH, *c* 0 0005), UV λ_{max} nm (log ε) 208 (3 97), IR ν_{max}^{Nujol} cm^{-1} 3420, 1770, 1740, 1710, 1504, 880, CD (MeOH) nm $\Delta\varepsilon_{230}$ −4 1, $\Delta\varepsilon_{295}$ −1 0, FDMS *m/z* 487 $[M+1]^+$, 410, 369, 327, 244, 190, 127, 85, ^{1}H NMR δ 0 65 (3H, *s*, 8-Me), 1 21 (3H, *s*, 4α-Me), 1 36 (3H, *s*, 13-Me), 1 44 (3H, *s*, 4β-Me), 1 6 (1H, *m*, H-12α), 1 73 (1H, *br dd*, $J=13$ and 8 Hz, H-11α), 1 85 (1H, *m*, H-11β), 1 95 (1H, *m*, H-12β), 2 64 (1H, *dd*, $J=15$ and 3 Hz, H_a-2), 2 79 (1H, *br d*, $J=12$ Hz, H-9), 2 92 (1H, *dd*, $J=15$ and 3 Hz, H_b-2), 3 12 (1H, *s*, H-5), 3 89 (1H, *d*, $J=3$ Hz, −OH), 4 15 (1H, *d*, $J=12$ 5 Hz, H_a-19), 4 16 1H, *s*, H-15), 4 28 (1H, *br d*, $J=12$ 5 Hz, H_b-19), 4 35 (1H, *br d*, $J=3$ Hz, H-7), 4 37 (1H, *br t*, $J=3$ Hz, H-1), 5 48 (1H, *br s*, H=17), 6 35 (1H, *m*, H-22), 7 43 (1H, *m*, H-23), 7 44 (1H, *m*, H-21) NOEs 13-Me/H-7 (5 8%), H-9 (6 1%), H-21 (4 2%), H-22 (6 1%), 4α-Me/H-1 (9.5%), 4β-Me/H-5 (12 0%), 8-Me/H-15 (18 4%), H_a-19 (3 6%), H_b-19 (11 3%)

Limonin diosphenol (**3**) Needles from Me_2CO, mp 273–278°, $[\alpha]_D^{24}$ −193° (Me_2CO, *c* 0 002), UV λ_{max} nm (log ε) 207 (3 90), 278 (3 90), IR ν_{max}^{Nujol} cm^{-1} 3450, 1740, 1690, 1660, 880, CD (MeOH) nm $\Delta\varepsilon_{208}$ −4 2, $\Delta\varepsilon_{275}$ +10 7, $\Delta\varepsilon_{320}$ −8 5, ^{1}H NMR δ 1 04 (3H, *s*, 8-Me), 1 16 (3H, *s*, 4β-Me), 1 49 (3H, *s*, 13-Me), 1 54 (3H, *s*, 4α-Me), 2 64 (1H, *dd*, J = 13 and 3 Hz, H-9), 2 84 (1H, *dd*, $J=17$ and 5 Hz, H_a-2), 2 91 (1H, *dd*, $J=17$ and 3 Hz, H_b-2), 4 07 (1H, *br t*, $J=3$ Hz, H-1), 4 12 (1H, *s*, H-15), 4 62 (1H, *d*, $J=13$ Hz, H_a-19), 4 66 (1H, *d*, $J=13$ Hz, H_b-19), 5.43 (1H, *s*, H-17), 6 23 (1H, *s*,-OH), 6 38 (1H, *m*, H-22), 7 40 (1H, *m*, H-23), 7 41 (1H, *m*, H-21)

Glaucin A Prisms from acetonitrile, mp > 310°, $[\alpha]_D^{15}$ −150° (MeCN, *c* 0 0005), UV λ_{max} nm (logε) 209 (3 79), IR ν_{max}^{Nujol} cm^{-1} 3450, 1765, 1740, 1700, 1503, 1110, 1050, 1010, 875, CD (MeOH) $\Delta\varepsilon_{228}$ −5 3, $\Delta\varepsilon_{297}$ −1 7, SIMS *m/z* 503 $[M+H]^+$, 645, 595, 553, 461, 277, 185, 93, 75, 57, ^{1}H NMR δ 0 61 (3H, *s*, 8-Me), 1 23 (3H, *s*, 4α-Me), 1 35 (3H, *s*, 13 − Me), 1 44 (3H, *s*, 4β-Me), 2 63 (1H, *dd*, $J=15$ and 3 Hz, H_a-2), 2.91 (1H, *dd*. $J=15$ and 3 Hz, H_b-2), 2 98 (1H, *br d*, $J=12$ Hz, H-9), 3 13 (1H, *s*, H-5), 3 87 (1H, *d*, $J=3$ Hz, -OH), 4 04 (1H, *m*, H-12β), 4 10 (1H, *d*, $J=12$ Hz, H_a-19), 4 15 (1H, *s*, H-15), 4 28 (1H, *d*, $J=12$ Hz, H_b-19), 4 34 (1H, *m*, H-7), 4 37 (1H, *d*, $J=7$ Hz, -OH), 4 45 (1H, *m*, H-1), 5 51 (1H, *s*, H-17), 6 46 (1H, *m*, H-22), 7 29 (2H, *m*, H-21 and H-23) (Found C, 62 12, H, 6 04% Calcd for $C_{26}H_{30}O_{10}$ C, 62 14, H, 6 02%)

α-Diketone **2** *from glaucin B* (**1**) To a MeOH soln (10 ml) of **1** (18 mg), 0 1 N Na_2CO_3 soln (3 ml) was added and the mixture stirred at room temp for 2 hr Work-up as usual gave 15 mg of crude product The product (8 mg) was stirred with Jones reagent in Me_2CO at room temp. for 1 hr Work-up as usual gave 6 mg of crude product, which was purified by column chromatography on silica gel and HPLC using a normal phase column to give **2** (3 mg) as pale yellow prisms, mp 282–286° (dec.) UV λ_{max} nm (logε) 208 (3 70), CD (MeOH) nm $\Delta\varepsilon_{211}$ −5 6 ($\pi-\pi^*$ of furan), $\Delta\varepsilon_{407}$ −1 0 (n-π^* of diketo) (Found C, 64 51, H, 5 81% Calcd for $C_{26}H_{28}O_9$ C, 64 45, H, 5 83%) To a CH_2Cl_2 soln of the hydrolysed product (7 mg) of **1**, pyridinium chlorochromate (30 mg) was added and stirred at room temp After 1 hr, MeOH (1 ml) was added and the mixture stirred for an additional 1 hr The crude product was chromatographed and purified by HPLC to give 4 mg of limonin diosphenol (**3**)

α-Diketone **2** *from rutaevin* [7] Rutaevin (7 mg) was treated with Jones reagent in Me_2CO at room temp in a similar manner described above to give **2** (4 mg) as pale yellow prisms, mp 283 286° (dec)

Limonin diosphenol (**3**) *from limonin* [8] Limonin (100 mg) suspended in *t*-butyl alcoholic *N* potassium *t*-butoxide (20 ml) was shaken with oxygen in a hydrogenation apparatus for 2 hr After addition of H_2O, the soln was acidified with 6 N HCl soln and extracted with $CHCl_3$ The $CHCl_3$ soln was washed with $NaHCO_3$ soln and H_2O, and then shaken with 4N NaOH soln Acidification of this extract with HCl soln gave a ppt which was extracted with $CHCl_3$ The $CHCl_3$ soln was washed with H_2O, and the solvent was removed Recrystallization from Me_2CO gave **3** (43 mg) as needles, mp 273–278°

Limonin diosphenol (**3**) *from rutaevin* Pyridinium chlorochromate (80 mg) was added to a stirred soln of rutaevin (44 mg) in CH_2Cl_2 After stirring over night at room temperature, MeOH was added and the mixture stirred for an additional 1 hr, and then the soln was evaporated *in vacuo* to yield a brown residue which was chromatographed over silica gel to give **3** (30 mg)

Biological activities (i) *Insect antifeedant activity* five limonoids, limonin, rutaevin, limonin diosphenol, glaucin A and glaucin B, were tested against the larvae of the pest insect *Spodoptera litura* Fab with the leaf disk method [11] Limonin and limonin diosphenol (**3**) showed the activity only at 1000 ppm concn (ii) *Antimicrobial activity* Effects of the compounds on growth of microorganisms were tested by the broth dilution method by Dr M Taniguchi (Osaka City University) Among the compounds rutaevin was active against a bacterium *Bacillus subtilis* at 100 μg/ml concn Test organisms bacterium, *S aureus*, *B subtilis*, *E coli* and *Ps aeruginosa* fungas, *Mucor mucedo*, *Rh chinensis*, *Asp niger* and *P crustosum* yiest, *S cerevisiae*, *C utilis*, *Schiz pombe* and *H anomala*

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