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DITERPENOIDS FROM *RABDOSIA JAPONICA*

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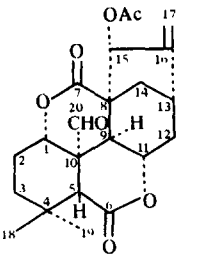
Key Word Index—*Rabdosia japonica*; Labiatae, structural determination; diterpenoids, glaucocalactone; X-ray diffraction

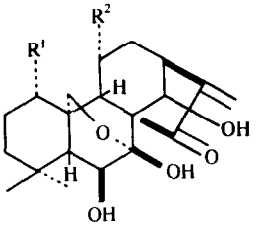
Abstract—From the leaves of *Rabdosia japonica*, a new *ent*-kaurene lactone type diterpenoid, glaucocalactone, along with β-sitosterol, oridonin and rosthorin A were isolated. The structure of glaucocalactone was elucidated on the basis of spectral data and X-ray crystallographic studies.

INTRODUCTION

The plants of *Rabdosia* species have been shown to contain some *ent*-kaurene type diterpenoids with cytotoxic activities [1–4]. In a continuation of our research work on diterpenoids, we have recently investigated *Rabdosia japonica* var. *glaucocalyx* collected from the southern Gansu Province of China From the ether extract of the leaves, four crystalline compounds were isolated One of the compounds is an *ent*-kaurene type lactone with a novel skeleton which we have named glaucocalactone (**1**), and the structure of which was elucidated as **1** on the basis of spectroscopic evidence and X-ray crystallographic studies The other three compounds were identified as oridonin (**2**) [5, 6], rosthorin A (**3**) [7] and β-sitosterol. None of these compounds has been found in this species previously



1



2 R^1=OH R^2=H

3 R^1=H R^2=OH

RESULTS AND DISCUSSION

Glaucocalactone (**1**), mp 318–320°, was isolated as colourless prisms. The mass spectral data and elemental analysis suggested its formula as $C_{22}H_{26}O_7$. The IR absorptions of **1** showed the presence of δ-lactone groups (1760, 1740 cm^{-1}), an aldehyde group (2750, 1720 cm^{-1}), a double bond (1660 cm^{-1}), and an acetoxy group (1725, 1240, 1040 cm^{-1}). The 400 MHz ^1H NMR spectrum (Table 1) showed the signals of two tertiary methyl groups, two methine protons attached to oxygen-substituted carbon atoms, one methine proton attached to a carbon atom bearing an acetoxyl group, an aldehyde proton, an exomethylene and an acetoxy group. The ^{13}C NMR spectrum (Table 1) of **1** showed the presence of two lactonic carbon atoms, one aldehyde carbon atom, one acetoxy group along with five methylene carbon atoms, three methine carbon atoms, three oxygen-bearing methine carbon atoms, two methyl carbon atoms and four quaternary carbon atoms

On comparison of the spectral data of **1** with those of closely related C-1α, C-11α and C-15α hydroxyl-substituted *ent*-kaurene compounds isolated from *Rabdosia* species [8–10], it appeared that glaucocalactone had a novel *ent*-kaurene lactone skeleton which is similar to that found in the *ent*-6,7-secokaurene diterpenoids [11].

The ^1H NMR signal of H-15 at δ5.92 (*t*, J=2.5 Hz) is clear evidence for the existence of a 15α acetoxy group while two signals at δ4.84 (*dd*, J=11.9, 4.2 Hz) and 5.00 (*dt*, J=11.3, 8 7 Hz) could be assigned on H-1β and H-11β. This established that the two lactone linkages were C-1, O-1α, C-7 and C-11, O-11α, C-6. Moreover, the singlet signal at δ10.00 indicated that the aldehyde group is attached to C-10

The structure and stereochemistry of glaucocalactone were finally determined by X-ray crystallographic studies.

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Table 1 ^{1}H and ^{13}C NMR data of compound **1**

H		C	δ
1	4 84 *dd* (11 9, 4 2)*	1	75 50 (CH)†
2	2 34 *dtd* (13 0, 11 9, 5 2)	2	23 94 (CH_2)
2	2 05 *dd* (13 0, 4.2)	3	38 47 (CH_2)
3	1 54 *m*—	4	32 22 (C)
3	1 62 *m*—	5	49 52 (CH)
5	2 86 *s*	6	169 38 (C)‡
9	2 43 *d* (11 3)	7	169 05 (C)‡
11	5 00 *dt* (11 3, 8 7)	8	49 72 (C)§
12	2 71 *ddd* (13 0, 8 7, 8 3)	9	39 49 (CH)
12	1 48 *dd* (13 0, 8 7)	10	48 23 (C)§
13	3 0 *dd* (8 3, 5 2)	11	68 62 (CH)
14	1 69 *dd* (12 5, 5 2)	12	36 24 (CH_2)
14	2 23 *d* (12 5)	13	36 59 (CH)
15	5 92 *t* (2 5)	14	31 47 (CH_2)
17	5 28 *dd* (2 5, 1 0)	15	80 77 (CH)
17	5 01 *br s*	16	152 19 (C)
20	10 00 *s*	17	111 50 (CH_2)
Me-19	0 93 *s*	18	22 24 (Me)
Me-18	1 10 *s*—	19	29 43 (Me)
OAc	1 99 *s*	20	202 26 (CH)
		21	171 55 (C)
		22	20 29 (Me)

The spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR in DMSO-d_6 solution at 25 using TMS as internal standard

* J (H_2) in parentheses

† Assignment made with aid of DEPT

‡,§ Assignments may be interchanged

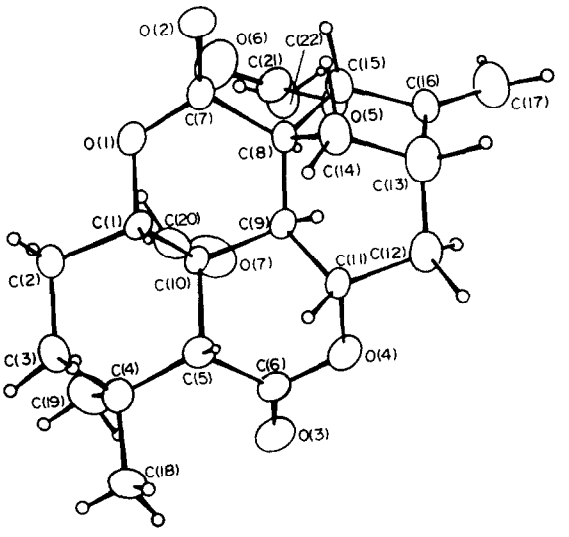

Fig 1

The structure of glaucocalactone was proved to be as shown in formula **1** The view of the molecule is given in Fig 1. The crystallographic data and summary of intensity data collection and structure refinement of **1** are shown in Table 2 The positional parameters were given in Table 3 All the crystallographic details are deposited with the Cambridge Crystallographic Data Centre

Table 2 Crystallographic data and summary of intensity data collection and structure refinement for compound **1**

Molecular formula	$C_{22}H_{26}O_7$
M_r	402 45
Space group	$P2_12_12_1$
T, K	298
a, Å	10 480 (2)
b, Å	11 875 (2)
c, Å	15 478 (3)
V, Å^3	1926 2 (8)
Z	4
d calc g/cm^3	1 383
Crystal dimensions, mm	0 6 × 0 5 × 0 1
Radiation wavelength Cu Kα, Å	1 542
Crystal decay	< 1
μ, cm^{-1}	8 139
F(000)	856
Scan mode	$\omega/2\theta$
Scan width in ω, °	(0 70 + 0 15 tanθ)
Aperture width, mm	(1 5 + 0 4 tanθ)
Aperture length, mm	4
Final acceptance limit	20 τ at 20 min
Maximum recording time, sec	60
Scan range, θ	2-55
No of reflections collected	1662
No of reflections observed (with $I > 3\,0\sigma I$)	1640
$R = \Sigma\|\|F_o\| - \|F_c\|\|/\Sigma\|F_o\|$	0 070
R_w	0 080

Table 3 Positional parameters and equivalent isotropic temperature factors of non-hydrogen atoms for compound **1**

Atom	X (× 10^4)	Y (× 10^4)	Z (× 10^4)	B_{eq} (× 10)
C(1)	11102(7)	225(6)	2101(4)	27(1)
C(2)	12073(8)	543(7)	1397(5)	38(1)
C(3)	13185(8)	−303(7)	1406(5)	39(2)
C(4)	13847(7)	−474(6)	2287(5)	31(2)
C(5)	12769(7)	−734(6)	2935(4)	26(1)
C(6)	13132(8)	−1104(6)	3846(5)	33(2)
C(7)	9088(7)	909(6)	2632(4)	27(1)
C(8)	9306(7)	166(6)	3439(4)	25(1)
C(9)	10690(7)	−151(5)	3683(4)	23(1)
C(10)	11703(7)	212(6)	3001(4)	23(1)
C(11)	10856(8)	−1420(6)	3896(4)	29(1)
C(12)	9872(9)	−1850(6)	4538(5)	40(2)
C(13)	8524(8)	−1382(7)	4304(5)	40(2)
C(14)	8475(8)	−944(6)	3365(5)	35(2)
C(15)	8600(7)	715(6)	4232(4)	27(1)
C(16)	8268(7)	−288(7)	4801(5)	34(2)
C(17)	7830(9)	−211(8)	5595(5)	46(2)
C(18)	14688(8)	−1555(7)	2222(6)	43(2)
C(19)	14687(8)	536(7)	2504(6)	46(2)
C(20)	12226(7)	1360(7)	3264(5)	35(2)
C(21)	9734(9)	2460(7)	4419(5)	38(2)
C(22)	10395(9)	3173(7)	5066(6)	45(2)
O(1)	10068(5)	1044(4)	2076(3)	33(1)
O(2)	8093(5)	1348(5)	2477(3)	39(1)
O(3)	14151(6)	−1073(5)	4171(4)	49(1)
O(4)	12105(5)	−1529(4)	4294(3)	37(1)
O(5)	9375(5)	1480(4)	4749(3)	33(1)
O(6)	9607(8)	2669(5)	3663(4)	66(2)
O(7)	12594(6)	1564(5)	3977(4)	55(1)

EXPERIMENTAL

Mps uncorr ^{1}H NMR: 400 MHz with TMS as an internal standard ^{13}C NMR 100 MHz with TMS as an internal standard IR KBr pellets MS direct inlet, 70 eV X-ray CAD-4 diffractometer, computations: PDP 11 computer The plant material was collected by Mr Zhang Guohang from the Liupan Mountain area of Gansu Province in July 1987 A voucher specimen has been deposited at the Herbarium of the Biology Department of Lanzhou University

Extraction and isolation The air-dried leaves of *R japonica* var *glaucocalyx* (2 1 kg) were treated with Et_2O for 5 days at room temp After removal of Et_2O, 80 g of syrup remained The syrup was mixed with 100 g silica gel (100 mesh), extracted with petrol (30–60°) to remove lipids and pigments, and then subjected to CC on silica gel (200–300 mesh) eluting a petrol–Me_2CO gradient (from 5 1 to 2 1) Three fractions were collected The first fraction was rechromatographed on silica gel eluted with C_6H_6–HOAc (7 1) to afford β-sitosterol (150 mg) Fraction 2 was subjected to further CC (CH_2Cl_2–Me_2CO, 8·1) to afford glaucocalactone (**1**) (17 mg), oridonin (**2**) (175 mg) and orsthorin A(**3**) (90 mg) Each component was further purified by repeated recrystallization from an appropriate solvent

Glaucocalactone (**1**) Colourless prisms from CH_2Cl_2, mp 318–320° (decomposed), $[\alpha]_D^{25}+55°$ ($CHCl_3$, *c* 0 30), UV λ_{max}^{MeOH} nm (log ε) 200 (2 3), IR ν_{max}^{KBr} cm^{-1} 2750, 1760, 1740, 1725, 1720, 1660, 1240, 1060, 1040, EIMS *m/z* (rel int) 402 1682 $[M]^+$ (9) ($C_{22}H_{26}O_7$ requires 402 1678), 360 $[M-CH_2CO]^+$ (55), 359 $[M-MeCO]^+$ (100), 331 $[M-CH_2CO-CHO]^+$ (12), 296 (13), 268 (12), 161 (28), 91 (25), 43 (95), ^{1}H and ^{13}C NMR Table 1 (Found C, 65 62, H, 6 50 $C_{22}H_{26}O_7$ requires C, 65 66, H, 6 51%)

Oridonin (**2**). Colourless needles from MeOH, mp 248–249°, $[\alpha]_D^{25}-43 5°$ (pyridine, *c* 0 80), UV λ_{max}^{EtOH} nm (log ε) 237 (3 94) IR ν_{max}^{KBr} cm^{-1} 3400–3200, 1700, 1640, 1095, 1080, 1060, EIMS *m/z* (rel int) 364 $[M]^+$ (8 6), 346 $[M-H_2O]^+$ (3 0), 328 $[M-2H_2O]^+$ (2 6), 149 (7 9), ^{1}H NMR (400 MHz): δ(pyridine-d_5) 1 13, 1 29 (each 3H, Me-18, Me-19), 1 46 (1H, *d*, *J* = 6 0 Hz, H-5β), 3 20 (1H, *d*, *J* = 9 3 Hz, H-13), 3 63 (1H, *dd*, *J* = 6, 11 Hz, H-6), 4 25 (1H, *t*, *J* = 7 0 Hz, H-1β), 4 40, 4 77 (each 1H, AB-system, *J* = 10 Hz, H-20a, H-20b), 5 32 (1H, *s*, H-14α), 5 50, 6 27 (each 1H, *s*, H-17a, H-17b), 6 00 (1H, *br s*, OH), 6 93 (1H, *d*, *J* = 11 Hz, 6-OH) (Found C, 65 89, H, 7 70 Calc for $C_{20}H_{28}O_6$ C, 65 92, H, 7 74%)

Rosthorin A(**3**) Colourless needles from MeOH, mp 257–259°, $[\alpha]_D^{25}-79°$ (pyridine, *c* 0 60), UV λ_{max}^{MeOH} nm (log ε) 236 (3.90), IR ν_{max}^{KBr} cm^{-1} 3470–3210, 1710, 1640, 1052, EIMS *m/z* (rel int) 364 $[M]^+$ (100), 346 $[M-H_2O]^+$ (8 5), 328 $[M-2H_2O]^+$ (10), 318 (11), 285 (8), 215 (10), 151 (58), 85 (65), ^{1}H NMR (400 MHz) δ(pyridine-d_5) 1 10, 1 22 (each 3H, *s*, Me-18, Me-19), 1 60 (1H, *d*, *J* = 6 Hz, H-5β), 1 94 (1H, *dd*, *J* = 9, 13 8 Hz, H-12β), 2 18 (1H, *d*, *J* = 8 7 Hz, H-9β), 3 05 (1H, *ddd*, *J* = 9 9, 13 8 Hz, H-12α), 3 27 (1H, *d*, *J* = 9 Hz, H-13), 4 17, 4 31 (each 1H, AB-system, *J* = 10 Hz, H-20a, H-20b), 4 26 (1H, *br s*, H-6), 4 43 (1H, *m*, H-11α), 5 20 (1H, *s*, H-14α), 5.51, 6 25 (each 1H, *s*, H-17a, H-17b), ^{13}C NMR (100 MHz) δ (pyridine-d_5) 30 70 (C-1), 18.99 (C-2), 41 48 (C-3), 34.18 (C-4), 59 30 (C-5), 73 69 (C-6), 98 45 (C-7), 60 73 (C-8), 61 57 (C-9), 37 87 (C-10), 62 95 (C-11), 41.89 (C-12), 44 32 (C-13), 73 84 (C-14), 209 26 (C-15), 152 38 (C-16), 119 42 (C-17), 33 36 (C-18), 22 42 (C-19), 67 26 (C-20) (Found C, 65 98, H, 7 72 Calc for $C_{20}H_{28}O_6$ C, 65 92, H, 7 74%)

β-Sitosterol (**4**) Colourless needles from petrol. Mp 139–140°, $[\alpha]_D^{25}-40°$ ($CHCl_3$, *c* 1 0), identical properties (mmp, IR) with an authentic sample of β-sitosterol.

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