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BENZOQUINONES, A HOMOISOFLAVANONE AND OTHER CONSTITUENTS FROM *POLYGONATUM ALTE-LOBATUM*

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Key Word Index—*Polygonatum alte-lobatum*; Liliaceae; rhizome; 1,4-benzoquinone; homoisoflavanone; polygonaquinone A; polygonaquinone B; gentrogenin glycoside.

Abstract—From the rhizomes of *Polygonatum alte-lobatum*, two new homologous series of 1,4-benzoquinones, polygonaquinones A and B, a novel homoisoflavanone, a new gentrogenin glycoside and 13 known compounds were isolated and characterized. The structures of the new compounds were determined as two homologous series of three 2,5-dihydroxy-3-methyl-6-alkyl-1,4-benzoquinones and three 2-hydroxy-3-methyl-6-alkyl-1,4-benzoquinones, with chain lengths C_{21} to C_{23}, and 4′,5,7-trihydroxy-6,8-dimethylhomoisoflavanone and gentrogenin 3-*O*-β-D-glucopyranosyl(1 → 2)-{β-D-xylopyranosyl(1 → 3)}-β-D-glucopyranosyl(1 → 4)-β-D-galactopyranoside.

INTRODUCTION

The rhizome of *Polygonatum alte-lobatum* Hayata, a Formosan endemic plant, has been used as a tonic drug in Taiwan. Various steroidal saponins and flavonoids have been reported from several *Polygonatum* species [1, 2], although no chemical work has been done on the rhizome of *P. alte-lobatum*. Continuing our studies on Formosan medicinal plants, we have isolated from the rhizomes of *P. alte-lobatum* two new homologous series of compounds, named polygonaquinones A (**1**) and B (**2**), respectively, a novel *C*-methylated homoisoflavanone (**3**), a new gentrogenin glycoside (**4**) and 13 known compounds. The present paper describes the isolation and structural characterization of the four new compounds.

RESULTS AND DISCUSSION

Compound **1** had similar UV maxima to 2,5-dialkyl-3,6-dihydroxy-*p*-benzoquinone [3]. Its IR spectrum showed bands for hydroxyl group at 3325 cm^{-1}, alkyl group at 2910 and 2850 cm^{-1} and a carbonyl group at 1610 cm^{-1}. The ^1H NMR spectrum of **1** showed signals at δ 0.87 (3H, *t*, Q-$(CH_2)_3(CH_2)_n$-CH_3), 1.26, 1.30 (nH, 2*s*, Q-$(CH_2)_3$-$(CH_2)_n$-CH_3), 1.45 (2H, *m*, Q-$CH_2CH_2CH_2(CH_2)_nCH_3$), 1.75 (2H, *m*, Q-CH_2-$CH_2CH_2(CH_2)_nCH_3$), 2.18 (3H, *s*, Q-CH_3) and 2.79 (2H, *t*, Q-$CH_2(CH_2)_2(CH_2)_n$-CH_3). These data confirm the existence of a methyl group and a long alkyl side chain linked to the quinonoid ring [4]. The ^{13}C NMR spectrum of **1** (Table 1) showed four enolic signals at δ 170.6 (C-1 and C-2) and 170.8 (C-4 and C-5) due to the tautomeric interconversion of the two equivalent forms of the 3,6-dihydroxy-*p*-benzoquinone [5] and a quinonoid methyl signal at δ 8.0. The EI-mass spectrum also appeared to suggest the existence of a long side chain, because it exhibits significant peaks differing one from the other by 14 mass units at *m/z* 476, 462 and 448, followed a group of three strong peaks at *m/z* 169, 168, 167 [4]. Based on the above evidence, polygonaquinone A (**1**) was characterized as a homologous series of three 2,5-dihydroxy-3-methyl-6-alkyl-1,4-benzoquinone with chain lengths C_{21} to C_{23} (**1**) [4].

After acetylation of **1**, two acetyl signals at δ 2.34 and 2.35 were observed in the ^1H NMR of **1** diacetate (**1a**), indicating the existence of two hydroxy groups in **1**. The ^{13}C NMR spectra of **1** and **1a** (Table 1) were assigned by comparison with those of 3,6-dihydroxythymoquinone [5]. The signals observed also supported the structure of **1**.

Compound **2** had similar UV maxima to 1,4-benzoquinone [6]. The IR spectrum of **2** showed bands for hydroxy group at 3250 cm^{-1} and carbonyl group at 1630 cm^{-1}. The ^1H NMR of **2** showed a quinonoid methyl signal at δ 1.82 (3H, *s*), a quinonoid proton signal at δ 6.40 (1H, *s*), an enolic signal at δ 6.85 (1H, *br s*), and long chain alkyl signals similar to those

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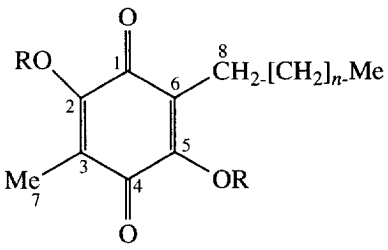
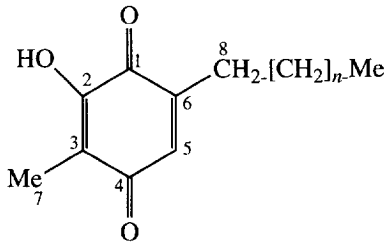


1 R = H, n = 19–21
1a R = AC, n = 19–21



2 n = 19–21

of **1**. The ^{13}C NMR spectrum (Table 1) showed two carbonyl signals at δ 183.6 and 188.0, and a quinonoid methyl signal at δ 7.9. The EI-mass spectrum of **2** also exhibits significant peaks differing one from the other by 14 mass units at m/z 460, 446 and 432, followed a group of three strong peaks at m/z 152, 153 and 154 [4]. Based on the above evidence, polygonaquinone B (**2**) was characterized as a homologous series of three 2-hydroxy-3-methyl-6-alkyl-1,4-benzoquinones with chain lengths C_{21} to C_{23} (**2**) [4]. The ^{13}C NMR spectrum of **2** was assigned by HMBC (Fig. 1), HMQC and comparison with those of **1**. The ^{13}C NMR, HMBC and HMQC spectra also supported the characterization of **2** as polygonaquinone B (**2**).

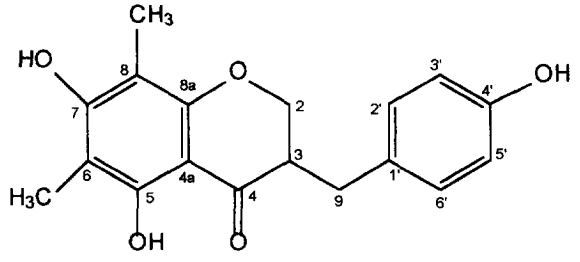
Compound **3**, $C_{18}H_{18}O_5$, had similar UV absorption maxima to 4′,5,7-trihydroxyhomoisoflavanone [7] and $AlCl_3$ and NaOAc induced bathochromic shifts suggested that **3** is a 5,7-dihydroxy-4′-oxygenated homoisoflavanone [8]. Its IR showed hydroxy absorption band at 3320 cm^{-1} and carbonyl at 1650 cm^{-1}. The ^1H NMR (CD_3OD) (Table 2) spectrum of **3** showed two methyl singlets at δ 1.97 and 1.98, signals of the -(2)-CH_2-(3)CH-(9)-CH_2- moiety at δ 4.22 (1H, *dd*, J = 4.2, 11.6 Hz), 4.07 (1H, *dd*, J = 6.6, 11.6 Hz), 2.74 (1H, *m*), 3.07 (1H, *dd*, J = 4.4, 13.6 Hz) and 2.62 (1H, *dd*, J = 10.4, 13.6 Hz) and signals of an aromatic AA′BB′ system (δ 6.74 and 7.05, J = 8.4 Hz; protons at H-2′, H-3′, H-5′ and H-6′) [7]. Based on the above evidence and the appearance of the hydroxytropylium fragment (m/z 107) in the mass spectrum, **3** can be characterized as 4′,5,7-trihydroxy-6,8-dimethylhomoisoflavanone (**3**). The ^{13}C NMR spectrum of **3** (Table 2) was assigned by ^1H-decoupled spectrum, DEPT pulse sequence, HMQC, HMBC (Fig. 2) and comparison of chemical shift values with those of corresponding data for 4′,5,7-trihydroxyhomoisoflavanone [7]. The ^{13}C NMR and 2D spectra of **3** also supported the characterization of **3**.

Compound **4**, mp 264–266°, was crystallized from the MeOH. Its IR showed strong absorption band for hydroxy group at 3350 cm^{-1} and characteristic bands for spiroketal moiety at 968, 943, 916 and 885 cm^{-1} [9]. It indicates that compound **4** is a spirostanol derivative. Acid hydrolysis yielded glucose, galactose and xylose, as detected by TLC, and gentrogenin, identified by comparison with an authentic sample. The FAB-MS spectrum of **4** showed a cationized molecular ion $[M + Na]^+$ at m/z 1070, indicating that **4** was a gentrogenin tetraoside having three hexose and one xylose moiety. The EIMS spectrum of **4** peracetate showed peaks due to the peracetylated terminal hexose (m/z 331) and pentose (m/z 259) [10]. It showed that **4** possessed the sugar sequence as shown in the structure. The ^1H NMR spectrum of **4** showed four anomeric proton signals at δ 4.89 (1H, *d*, J = 7.6 Hz), 5.21 (1H, *d*, J = 7.6 Hz) and 5.26 (2H, *d*, J = 6.4 Hz) confirming the presence of four sugars in the structure of **4** and a broad doublet at δ 5.60 (1H, *d*, J = 5.6 Hz, H-6). The ^{13}C NMR spectrum (Table 3) showed four anomeric carbon signals at δ 102.7, 104.8, 104.9 and 105.2 and all the sugar carbon chemical shifts were almost superimposable on those of corresponding carbons in 26-*O*-β-D-glucopyranosyl-22-*O*-methyl-25(*S*)-furost-5-ene-3β,26-di-ol-3-*O*-β-lycotetraoside [1]. This evidence identifies structure **4** as shown.

EXPERIMENTAL

Extraction and isolation. Fresh rhizomes of *Polygonatum alte-lobatum* Hayata (20 kg) was collected at Pin-Tung Hsien, Taiwan, R.O.C., during October 1995. A voucher specimen deposited in the authors' laboratory. The fresh rhizomes were chipped and extracted with MeOH at room temp. in a closed container several times. The extract was chromatographed on a silica gel column. Elution with CH_2Cl_2 yielded diosgenin, gentrogenin, **1**, **2**, sitosterol and stigmasterol, elution with CH_2Cl_2–MeOH (8:2) yielded *N*-(*p*-coumaroyl)tyramine, 2′-hydroxyflavone, **3**, quercetin, sitosteryl-3-*O*-β-D-glucoside and **4**, elution with CH_2Cl_2–MeOH (6:4) yielded 2-L-pyrrolidone-5-carboxylic acid, allantoin, urea and elution with CH_2Cl_2–MeOH (2:8) yielded sucrose and methyl-α-D-fructofuranose. The characterization of known compounds was achieved by spectral methods.

Compound **1**. Orange powder (EtOAc), mp 121–123°, UV λ_{max}^{MeOH} nm: 210 (log ε 4.10), 283 (log ε 4.31), 402 (log ε 2.30), 420 (log ε 2.01). ^1H NMR ($CDCl_3$): see text. ^{13}C NMR ($CDCl_3$): see Table 1. EIMS (direct inlet) 70 eV, m/z (rel. int.): 476 $[M]^+$ (13), 426 (3), 449 (19), 448 (60), 169 (62), 168 (100), 167 (42), 156 (28), 139 (17). Acetate **1a**, ^1H NMR ($CDCl_3$): δ 0.88(3H, *t*), 1.24–1.41 (42H, *br s*), 1.96 (3H, *s*, 7-Me), 2.34 (3H, *s*,



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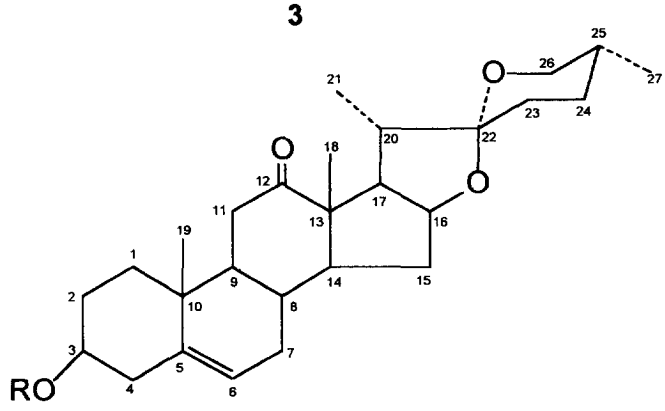

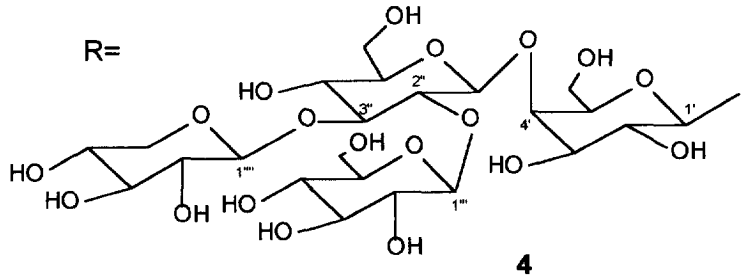

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Table 1. ^{13}C NMR chemical shift assignment of **1**, **1a** and **2**

C	**1** (Pyridine-d_5)	**1a** ($CDCl_3$)	**2** ($CDCl_3$)*
1	170.6	179.3[a]	183.66
2	170.6	148.9[b]	151.3
3	112.7	135.8	117
4	170.8	180.0[a]	188
5	170.8	149.0[b]	134.3
6	117.3	131.9	145
7-CH_3	8.0	9.2	7.9
CH_2	28.9, 29.6, 29.9, 30.0, 30.2, 32.1	28.3, 29.2, 29.4, 29.6, 29.7, 31.9	27.6, 28.2, 29.2, 29.3, 29.5, 29.6, 29.7, 31.9
$CH_2CH_2CH_3$	23	23.8	22.7
CH_2CH_3	22.9	22.7	22.7
CH_3	14.2	14.1	14.1
$OCOCH_3$		20.2, 20.3	
$OCOCH_3$		167.6, 167.9	

The number of protons directly attached to each carbon was verified by DEPT pulse sequence.
*These signals were assigned by HMQC and HMBC spectra.
[a,b]These signals may be interchangeable in each column.

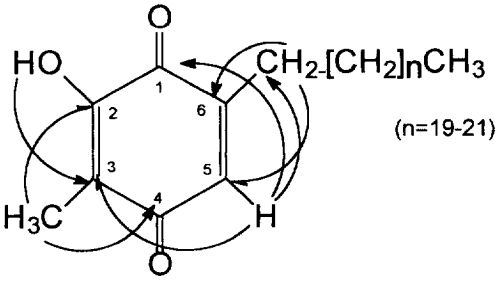

Fig. 1.

OAc), 2.35 (3H, *s*, OAc), 2.39 (2H, *t*). ^{13}C NMR ($CDCl_3$): Table 1.

Compound **2**. Orange powder ($CHCl_3$–MeOH), mp 70°, UV λ_{max}^{MeOH}: 210 (log ε 4.01), 268 (log ε 4.12), 402 (log ε 2.13). ^{1}H NMR ($CDCl_3$): see text. ^{13}C NMR ($CDCl_3$): Table 1. EIMS (direct inlet) 70 eV, *m/z* (rel. int.): 460 $[M]^+$ (27), 446 (5), 432 (64), 154 (28), 153 (44), 152 (14), 124 (11), 85 (15), 83 (11), 71 (24), 69 (17), 57 (67), 55 (36), 43 (100).

Compound **3**. Pale yellow needles ($CHCl_3$–MeOH), mp 102–103°, $[\alpha]_D^{25}$ − 91.7° (MeOH, *c* = 0.05). UV λ_{max}^{MeOH} nm: 238 (log ε 4.29), 291 (log ε 4.41), 338 (sh). ^{1}H NMR ($CDCl_3$): see text. ^{13}C NMR ($CDCl_3$): Table 2. EIMS (direct inlet) 70 eV, *m/z* (rel. int.): 314 $[M]^+$ (45), 209 (24), 208 (70), 207 (67), 190 (2), 181 (16), 165 (9), 152 (13), 133 (8), 108 (17), 107 (100), 95 (7), 83 (13), 77 (26). HRMS Calc. for $C_{18}H_{18}O_5$: 314.1154; found: 314.1152.

Compound **4**. Powder (MeOH), mp 264–266°, $[\alpha]_D^{25}$ − 40.0° (MeOH, *c* = 0.01), ^{1}H NMR (pyridine-d_5): δ 0.69 (3H, *d*, *J* = 5.2 Hz, Me-27), 0.89 (3H, *s*, Me-19), 1.09 (3H, *s*, Me-18), 1.36 (3H, *d*, *J* = 6.8 Hz, Me-21), 4.89 (1H, *d*, *J* = 7.6 Hz, H-1′), 5.21 (1H, *d*, *J* = 7.6 Hz, H-1‴), 5.26 (2H, *d*, *J* = 6.4 Hz, H-1″ and 1⁗), 5.60 (1H, *d*, *J* = 5.6 Hz, H-6); ^{13}C NMR: Table 3. FAB MS (positive mode) *m/z* (rel. int.): 1070 $[M + Na]^+$ (3), 591 (8), 411 (33), 251 (17), 145 (48), 139 (60), 91 (100), 55 (95). Compound **4** was hydrolysed with 3 N HCl–MeOH to yield gentrogenin whose identity was confirmed by direct comparison of mp, IR, NMR and MS spectra with those of an authentic sample. The sugar portion was examined by TLC [$CHCl_3$–MeOH–Me_2CO–H_2O (3:3:3:1) on silica gel] to detect methyl galactopyranoside (R_f = 0.30), methyl glucopyranoside (R_f = 0.33) and methyl-α,β-D-xylopyranoside (R_f = 0.50). Peracetate (**4a**), powder, ^{1}H NMR ($CDCl_3$): δ 0.79 (3H, *d*, *J* = 6.4 Hz, Me-27), 1.07 (3H, *d*, *J* = 7.2 Hz, Me-21), 1.10 (3H, *s*, Me-19), 1.25 (3H, *s*, Me-18), 4.83 (1H, *d*, *J* = 7.6 Hz, H-1′), 5.16 (1H, *d*, *J* = 7.2 Hz, H-1‴), 5.31 (2H, *d*, *J* = 6.4 Hz, H-1″, 1⁗), 5.35 (1H, *d*, *J* = 5.6 Hz, H-6). EIMS (direct inlet) 70 eV, *m/z* (rel. int.): no molecular ion,

Table 2. ^{13}C NMR chemical shift assignment of **3** (in CD_3OD)

C		C	
2	70.0	4a	102.5
3	48.1	8a	159.1
4	199.8	9	33.2
5	160.6	1′	130.4
6	104.9	2′	131.1
7	164.8	3′	116.4
8	103.8	4′	157.1
6-Me	7.9	5′	116.4
8-Me	7.5	6′	131.1

The number of protons directly attached to each carbon was verified by DEPT pulse sequence. These signals were assigned by HMQC and HMBC spectra.

Table 3. ^{13}C NMR chemical shift assignment of **4** (in pyridine-d_5)

C/aglycone		C/aglycone		C/aglycone	
1	36.9	10	37.5	19	18.8
2	29.8	11	37.5	20	42.6
3	77.8	12	212.6	21	13.9
4	39.0	13	54.9	22	109.3
5	140.8	14	55.9	23	31.6
6	121.4	15	31.7	24	29.2
7	31.8	16	79.7	25	30.5
8	30.8	17	54.0	26	66.9
9	52.2	18	15.9	27	17.3

C/sugar		C/sugar		C/sugar		C/sugar	
1′	102.7	1″	104.8	1‴	104.9	1⁗	105.2
2′	73.2	2″	81.4	2‴	75.3	2⁗	75.6
3′	75.1	3″	86.6	3‴	78.8	3⁗	77.6
4′	79.9	4″	70.7	4‴	71.0	4⁗	70.5
5′	76.3	5″	78.7	5‴	77.7	5⁗	67.4
6′	60.5	6″	62.5	6‴	63.0		

The number of protons directly attached to each carbon was verified by DEPT pulse sequence.

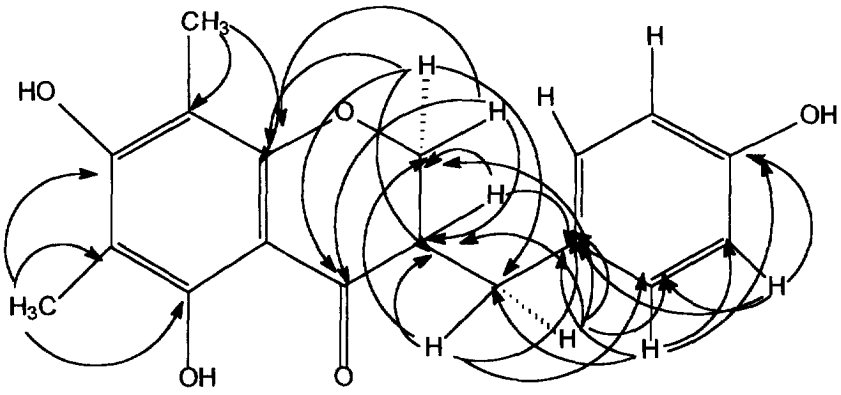

Fig. 2.

331 (100), 259 (5), 211 (9), 169 (74), 139 (10), 109 (45), 97 (39), 85 (45), 69 (54), 57 (80).

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REFERENCES

1. Son, K. H., Do, J. C. and Kang, S. S., *Journal of Natural Products*, 1990, **53**, 333.
2. Morita, N., Arisawa, M. and Yoshikawa, A., *Yakugaku Zasshi*, 1976, **96**, 1180.
3. Ogawa, H. and Natori, S., *Chemical and Pharmaceutical Bulletin*, 1965, **13**, 511.
4. Alves, A. C., Moreira, M. M., Paul, M. I. and Cruz Costa, M. A., *Phytochemistry*, 1992, **31**, 2825.
5. Joseph-Nathan, P., Martinez, E., Rojas, M. and Santillan, R. L., *Journal of Natural Products*, 1987, **50**, 860.
6. Scott, A. I., *Interpretation of the Ultraviolet Spectra of Natural Products*, 1st edn. Pergamon Press, Oxford, 1964, p. 123.
7. Adinolfi, M., Barone, G., Lanzetta, R., Laonigro, G., Mangoni, L. and Parrilli, M., *Phytochemistry*, 1985, **24**, 624.
8. Mabry, T. J. and Markham, K. R., in *The Flavonoids*, eds J. B. Harborne, T. J. Mabry and H. Mabry, Chapter 2. Academic Press, New York, 1975.
9. Ono, M., Shogama, K. and Nohara, T., *Shoyakugaku Zasshi*, 1988, **42**, 135.
10. Gan, K. H., Lin, C. N. and Won, S. J., *Journal of Natural Products*, 1993, **56**, 15.