The Structures of Rabdosianin A, B, and C, Three New Diterpenoids from *Rabdosia shikokianus* Hara

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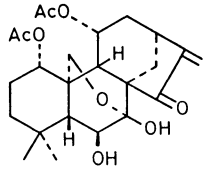
Three new bitter diterpenoids, rabdosianin A, B, and C, have been isolated from the leaves of *Rabdosia shikokianus* Hara, and their structures, determined by spectroscopic and chemical methods. Complete assignments of the ^1H and ^{13}C NMR data are described.

The isolation of four diterpenoids: oridonin (**1**),[1] shikokianin (**2**),[1] shikokianidin (**3**),[2] and shikodonin (**4**),[3] from the leaves of *Rabdosia shikokianus* Hara[4] has already been reported. Further investigation of the bitter principles from this plant has led to the isolation of five additional diterpenoids: effusanin B (**5**),[5] longikaurin E (**6**),[6] and three new *ent*-kaurenoids. In this paper, we will deal with the isolation and structures of these new compounds, designated as rabdosianin A, B, andC.



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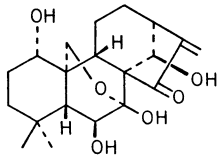

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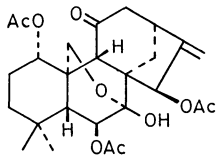

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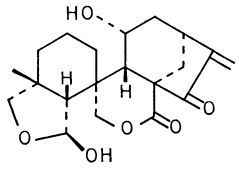

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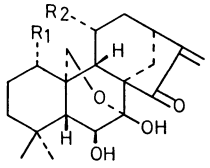
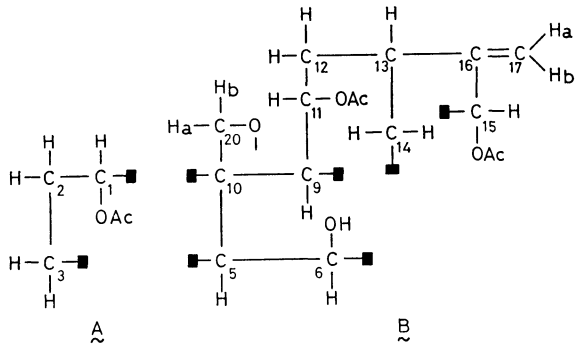


5 R_1=OAc, R_2=H
6 R_1=H, R_2=OAc

Rabdosianin A (**7**), $C_{26}H_{36}O_9$, mp 150—153 °C, $[\alpha]_D^{20}$ −50° (*c* 0.21, $CHCl_3$), was isolated as white needles in 0.053% yield from the ether extract of the dried leaves by extensive column chromatography. It had IR absorptions indicative of hydroxyl (3540, 3440), acetoxyl (1740, 1235), and *exo*-methylene (1660 and 902 cm^{-1}) groups. The ^1H NMR spectrum at 400 MHz showed signals due to two tertiary methyl groups at δ 1.08 and 1.14, three acetyl groups at δ 1.94, 2.10, and 2.11, one secondary hydroxyl group at δ 2.48 (d, J=2.4 Hz), one tertiary hydroxyl group at δ 3.17, one methine proton on an oxygenated carbon atom at δ 3.96 (dd, J=7.8 and 2.4 Hz), one ether type methylene group at δ 4.12 (dd, J=8.9 and 1.0 Hz) and 4.50 (dd, J=8.9 and 1.6 Hz), three methine protons attached to carbon atoms bearing an acetoxyl group at δ 4.82 (dd, J=4.1 and 3.5 Hz), 4.87 (dd, J=10.7 and 4.6 Hz), and 5.63 (dd, J=1.9 and 1.0 Hz), and one terminal methylene group at δ 4.86 (br s) and 5.03 (dd, J=1.9 and 1.0 Hz). In addition, extensive ^1H NMDR studies, summarized in Table 1, revealed the presence of the partial structures (**A**) and (**B**), the latter showing the following long-range couplings: $J_{H_5,H_{20b}}$=1.6 Hz, $J_{H_9,H_{20a}}$=1.0 Hz, $J_{H_{13},H_{17a}}$=1.9 Hz, $J_{H_{13},H_{17b}}$=1.0 Hz, $J_{H_{15},H_{17a}}$=1.0 Hz, and $J_{H_{15},H_{17b}}$=1.9 Hz.



A **B**

The spectral data and the analogy with the congeners isolated from *Rabdosia* species[1,2] suggested the structure **7** for rabdosianin A. The proposed structure was also supported by the ^{13}C NMR data at 100 MHz (Table 2), which showed the presence of one hemiacetal group (δ 96.5), four methylene groups, three methine groups, and three quaternary carbon atoms. The α-configuration of two acetoxyl groups at C_1 and C_{11} was evident from the J values of H_1 and H_{11} signals. Namely, the large axial-axial coupling (J=10.7 Hz) and the small axial-equatorial coupling (J=4.6 Hz) of the former signal indicated the axial nature of the corresponding proton. Hence, the acetoxyl group at C_1 must be α-oriented. The small J values of the latter signal ($J_{H_9,H_{11}}$=4.1 Hz, $J_{H_{11},H_{12\beta}}$= 3.5 Hz, and $J_{H_{11},H_{12\alpha}}\approx$0 Hz) revealed that the di-

Table 1. ^{1}H NMR spectra at 400 MHz of rabdosianin A (**7**), B (**10**), and C (**12**)[a]

	7	**10**	**12**
$H_{1\alpha}$			1.87 (ddd, 13.0, 1.9, 1.9)
$H_{1\beta}$	4.87 (dd, 10.7, 4.6)	4.89 (dd, 11.5, 5.0)	1.43 (m)
$H_{2\alpha}$	1.39 (m)	*ca.* 1.40 (m)	*ca.* 1.55 (m)
$H_{2\beta}$	1.77 (m)	1.81 (m)	*ca.* 1.55 (m)
$H_{3\alpha}$	1.37 (br d, 12.4)	*ca.* 1.45 (m)	*ca.* 1.55 (m)
$H_{3\beta}$	1.45 (ddd, 12.4, 10.0, 3.0)	*ca.* 1.40 (m)	*ca.* 1.20 (m)
H_5	1.68 (dd, 7.8, 1.6)	1.96 (dd, 8.4, 1.9)	1.38 (dd, 4.6, 1.1)
H_6	3.96 (dd, 7.8, 2.4)	5.33 (d, 8.4)	5.17 (d, 4.6)
H_9	2.15 (dd, 4.1, 1.0)	2.19 (dd, 3.8, 1.2)	2.26 (dd, 2.4, 2.2)
H_{11}	4.82 (dd, 4.1, 3.5)	4.82 (dd, 4.1, 3.8)	
$H_{12\alpha}$	2.31 (dd, 15.7, 9.5)	2.32 (dd, 15.7, 9.2)	3.11 (m)
$H_{12\beta}$	1.65 (dd, 15.7, 3.5)	1.62 (dd, 15.7, 4.1)	3.13 (m)
H_{13}	2.65 (dddd, 9.5, 4.3, 1.9, 1.0)	2.64 (dddd, 9.2, 4.9, 1.4, 0.8)	2.94 (dddd, 4.6, 4.4, 1.0, 1.0)
$H_{14\alpha}$	2.49 (d, 12.4)	2.47 (d, 12.4)	2.20 (d, 12.4)
$H_{14\beta}$	1.63 (dd, 12.4, 4.3)	1.72 (dd, 12.4, 4.9)	1.63 (dd, 12.4, 4.6)
H_{15}	5.63 (dd, 1.9, 1.0)	5.66 (dd, 2.4, 2.2)	4.53 (ddd, 1.9, 1.9, 1.1)
H_{17a}	4.86 (br s)	4.91 (dd, 2.2, 1.4)	5.18 (br s)
H_{17b}	5.03 (dd, 1.9, 1.0)	5.05 (dd, 2.4, 2.2)	5.25 (br s)
H_{20a}	4.12 (dd, 8.9, 1.0)	4.16 (dd, 9.5, 1.2)	4.03 (dd, 9.5, 2.2)
H_{20b}	4.50 (dd, 8.9, 1.6)	4.55 (dd, 9.5, 1.9)	4.16 (dd, 9.5, 1.1)
Me_{18}	1.08 (s)	0.89 (s)	0.85 (s)
Me_{19}	1.14 (s)	1.16 (s)	1.14 (s)
6-OH[b]	2.48 (d, 2.4)		
7-OH[b]	3.17 (s)	3.43 (s)	3.19 (s)
15-OH[b]			3.30 (d, 1.9)
OAc	2.11, 2.10, 1.94	2.16, 2.09, 2.06, 1.94	2.18

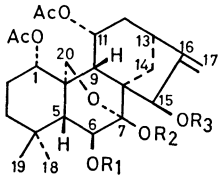
a) The spectra were determined at 25 °C in $CDCl_3$ solutions with TMS as an internal standard. Multiplicity and J values (in Hz) are in parentheses. b) These signals disappeared on addition of D_2O.

hedral angles between H_9 and H_{11}, and H_{11} and $H_{12\beta}$, are both close to 45°, and that between H_{11} and $H_{12\alpha}$ is roughly 90°. Thus, the acetoxyl group at C_{11} is α-oriented.

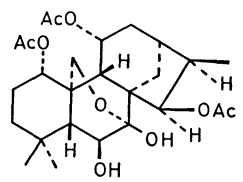
The catalytic hydrogenation of **7** afforded a dihydro compound (**8**), $C_{26}H_{38}O_9$, mp 252—253 °C, as the major product. Since the hydrogenation of the C_{16} double bond is assumed to occur from the less hindered α-side, the newly introduced secondary methyl group must be β-oriented.[2] The ^{1}H NMR spectrum of **8** displayed a signal of H_{15} as a doublet at δ 5.20 (J=10.4 Hz), the large J value of which defined the configuration of the acetoxyl group at C_{15} as β.[2] The β-configuration for the secondary hydroxyl group at C_6 is common to all *ent*-kaurenoids from *Rabdosia* species which have the same functional groups in the B-ring as **7**.

Finally, the structure was confirmed by the chemical correlation of **7** with **2**. Thus, the partial hydrolysis of **7** with methanol–0.1 M (1M=1 mol dm^{-3}) sodium hydroxide solution (1:1) gave a triol (**9**), $C_{24}H_{34}O_8$, mp 254—256 °C, which was found to be identical with the compound derived from **2** by the reduction with sodium borohydride.

Rabdosianin B (**10**), $C_{28}H_{38}O_{10}$, mp 215 °C (dec), $[\alpha]_D^{20}$ −70° (c 0.39, $CHCl_3$), was isolated as colorless prisms in 0.004% yield and exhibited spectral data quite similar to those of **7**, except for the following



7 R_1=R_2=H, R_3=Ac
9 R_1=R_2=R_3=H
10 R_1=R_3=Ac, R_2=H
11 R_1=R_2=R_3=Ac



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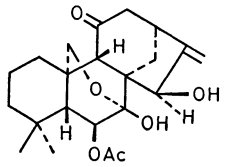

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observation: the IR spectrum did not show the presence of a secondary hydroxyl group and, instead, the ^{1}H NMR spectrum (Table 1) revealed the signals due to four acetoxyl groups, one more than those of **7**. These facts, and the appearance of the H_6 signal at a lower field (δ 5.33) as a doublet (J=8.4 Hz) suggested that an acetoxyl group took the place of the secondary hydroxyl group at C_6 of **7**. From the evidence outlined above and the pertinent ^{13}C NMR data (Table 2), we assigned the structure **10**

Table 2. ^{13}C chemical shifts of rabdosianin A (**7**), B (**10**), and C (**12**)[a]

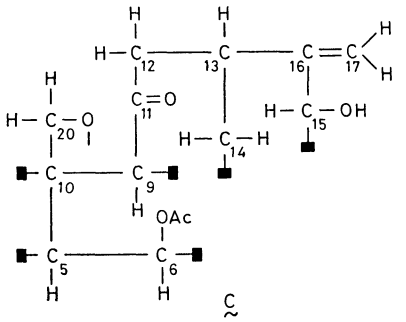
Carbon	7	10	12
1	76.9 d	76.7 d	41.1 t
2	25.3 t	25.2 t	18.5 t
3	38.9 t	38.7 t	41.4 t
4	33.4 s	33.5 s	33.9 s
5	53.5 d	52.5 d	53.3 d
6	74.1 d	75.7 d	74.1 d
7	96.5 s	95.9 s	96.1 s
8	50.7 s	50.6 s	50.9 s
9	48.4 d	48.4 d	58.3 d
10	40.7 s	41.1 s	37.6 s
11	69.6 d	69.5 d	199.0 s
12	39.9 t	39.9 t	50.3 t
13	35.1 d	35.4 d	37.7 d
14	26.7 t	26.7 t	29.8 t
15	74.9 d	75.2 d	75.2 d
16	157.0 s	157.1 s	151.6 s
17	109.5 t	110.0 t	109.6 t
18	22.6 q	22.7 q	21.5 q
19	33.8 q	33.5 q	32.1 q
20	65.5 t	65.4 t	68.1 t
$\underline{C}OCH_3$	171.7 s	173.8 s	170.2 s
	170.8 s	170.8 s	
	170.0 s	170.5 s	
		170.1 s	
$CO\underline{C}H_3$	22.2 q	22.3 q	21.5 q
	21.9 q	22.1 q	
	21.8 q	22.0 q	
		21.3 q	

a) The spectra were measured at 100.61 MHz in $CDCl_3$ solutions and the shifts are given in ppm (δ) relative to the internal TMS. Assignments were made by off-resonance and selective proton-noise decouping technique.

for rabdosianin B.

Final confirmation of the structure was achieved by the chemical correlation of **10** with **7**. The acetylation of **10** with acetic anhydride–boron trifluoride etherate gave a pentaacetate (**11**), $C_{30}H_{40}O_{11}$, mp 182—183 °C, which was also obtained by a similar treatment from **7**.

Rabdosianin C (**12**), $C_{22}H_{30}O_6$, mp 222—225 °C, $[\alpha]_D^{20}$ −170° (*c* 0.15, $CHCl_3$), was obtained as colorless flakes in 0.003% yield and showed IR absorptions attributable to hydroxyl (3520, 3320), carbonyl (1755), and *exo*-methylene (3080, 1660, and 905 cm^{-1}) groups. The ^1H NMR spectrum exhibited signals due to two tertiary methyl groups at δ 0.85 and 1.14, one acetyl group at δ 2.18, one tertiary hydroxyl group at δ 3.19, one secondary hydroxyl group at δ 3.30 (d, J=1.9 Hz), one ether type methylene group at δ 4.03 (dd, J=9.5 and 2.2 Hz) and 4.16 (dd, J=9.5 and 1.1 Hz), one methine proton on an oxygenated carbon atom at δ 4.53 (ddd, J=1.9, 1.9, and 1.1 Hz), one methine proton attached to a carbon atom bearing an acetoxyl group at δ 5.17 (d, J=4.6 Hz), and one terminal methylene group at δ 5.18 and 5.25 (each br s). The ^{13}C NMR data (Table 2) revealed the presence of one acetal group (δ 96.1) and one ketonic carbon atom (δ 199.0) together with five methylene groups, three methine groups, and three quaternary carbon atoms. In addition, the presence of the partial structure (**C**) was revealed by extensive ^1H NMDR studies, the results of which are tabulated in Table 1. A long-range coupling across a ketonic group ($J_{H_9,H_{12\beta}}$=2.4 Hz) was observed in this system. Thus, all of the ten oxygen atoms in **12** can be characterized by the functional groups in the partial sturcture (**C**) and a hemiacetal group.



From the aforementioned data, it would be quite reasonable to assign the *ent*-kaurenoid structure for rabdosianin C, in which the partial structure (**C**) would form the B, C, and D-rings. Observation of about 10% N. O. E. on H_{15}, upon irradiation of $H_{14\beta}$, defined the configuration of the secondary hydroxyl group at C_{15} as β. The magnitude of the coupling constant between H_6 and H_5 (J=4.6 Hz) indicated that the acetoxyl group at C_6 is β-oriented.[2] From this evidence, we propose the structure **12** for rabdosianin C.

Experimental

All mps were determined on a Mitamura micro melting point apparatus and are uncorrected. The IR spectra were recorded on a JASCO model IRA-1 spectrophotometer. The ^1H and ^{13}C NMR spectra were taken on a Bruker WH-400 instrument in $CDCl_3$ solutions with TMS as an internal standard unless otherwise stated. A Union Gikken apparatus, model PM-101, was used for the measurement of the rotations.

Isolation. The dried leaves (5.2 kg) of *Rabdosia shikokianus* Hara were extracted with ether (50 l). The ether solution was treated with activated charcoal (50 g) and evaporated to dryness. The residue (210 g) was subjected to chromatography over silicic acid (1.5 kg) eluting with $CHCl_3$–MeOH mixtures, with MeOH increasing from 0 to 10%. Elution with 2% MeOH in $CHCl_3$ gave a fraction (15 g) which was rechromatographed over silicic acid (300 g), using 40% ethyl acetate in petroleum ether as eluent, to yield rabdosianin C (**12**) (115 mg) and B (**10**) (250 mg). The material (28 g) which was eluted with 5% MeOH in $CHCl_3$ was rechromatographed over silicic acid (600 g), using 50% ethyl acetate in petroleum ether as eluent, to give effusanin B (**5**) (203 mg) and longikaurin E (**6**) (71 mg). The material (22 g) eluted with 10% MeOH in $CHCl_3$ was rechromatographed over silicic acid (600 g), using 70% ethyl acetate in petroleum ether as eluent, to afford rabdosianin A (**7**) (2.70 g).

Rabdosianin A (7). The crude material was recrystallized from ether to give fine needles (1.60 g), mp 150—153 °C; $[\alpha]_D^{20}$ −50° (*c* 0.21, $CHCl_3$); IR (Nujol) 3540, 3440, 1740, 1660, 1235, and 902 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); MS *m/e* 492 (M^+), 450, 432 (M^+-AcOH), 390, 372 ($M^+-2AcOH$), 330, and 312 ($M^+-3AcOH$). Found: C, 62.91; H, 7.21%. Calcd for $C_{26}H_{36}O_9$: C, 63.40; H, 7.37%.

Rabdosianin B (10). The crude solid was recrystallized from ether to yield colorless prisms (156 mg), mp 215 °C (dec); $[\alpha]_D^{20}$ −70° (*c* 0.39, $CHCl_3$); IR (Nujol) 3530, 3080, 1740, 1720, 1660, 1240, and 895 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); MS *m/e* 534 (M^+), 474 (M^+-AcOH), 432, 414 ($M^+-2AcOH$), 372, 354 ($M^+-3AcOH$), 312, and 294 ($M^+-4AcOH$). Found: C, 62.52; H, 7.06%. Calcd for $C_{28}H_{38}O_{10}$: C, 62.91; H, 7.17%.

Rabdosianin C (12). The crude substance was recrystallized from EtOH to afford colorless flakes (75 mg), mp 222—225 °C; $[\alpha]_D^{20}$ −170° (*c* 0.15, $CHCl_3$); IR (Nujol) 3520, 3320, 3080, 1755, 1660, 1245, and 905 cm^{-1}; ^1H NMR (see Table 1); ^{13}C NMR (see Table 2); MS *m/e* 372 (M^+-H_2O), 330 (M^+-AcOH), 312, 284, and 266. Found: C, 67.58; H, 7.79%. Calcd for $C_{22}H_{30}O_6$: C, 67.67; H, 7.74%.

Hydrogenation of **7**. **7** (100 mg) and 10% Pd-C (40 mg) were stirred in EtOH (10 ml) under a hydrogen atmosphere at room temperature for 16 h. The catalyst was removed by filtration, and the filtrate was evaporated. The residue was subjected to chromatography over silicic acid (10 g). Elution with ether gave dihydrorabdosianin A (**8**) (50 mg) which was recrystallized from aqueous MeOH to yield colorless plates (30 mg), mp 252—253 °C; IR (Nujol) 3570, 3460, 1740, 1735, 1720, and 1255 cm^{-1}; ^1H NMR δ 0.75 (3H, d, J=7.6 Hz, 17-Me), 1.05 and 1.12 (3H each, s, 18- and 19-Me), 1.95, 2.06, and 2.09 (3H each, s, 3Ac), 2.30 (1H, d, J=2.1 Hz, 6-OH), 2.87 (1H, s, 7-OH), 3.87 (1H, dd, J=7.3 and 2.1 Hz, H_6), 4.10 (1H, br d, J=8.9 Hz, H_{20a}), 4.51 (1H, dd, J=8.9 and 1.5 Hz, H_{20b}), 4.81 (1H, dd, J=3.6 and 3.4 Hz, H_{11}), 4.87 (1H, dd, J=10.7 and 4.6 Hz, H_1), and 5.20 (1H, d, J=10.4 Hz, H_{15}). Found: C, 63.08; H, 7.57%. Calcd for $C_{26}H_{38}O_9$: C, 63.14; H, 7.75%.

Partial Hydrolysis of **7**. A solution of **7** (150 mg) in MeOH (30 ml) and 0.1 M NaOH solution (30 ml) was allowed to stand in a refrigerator for 14 h to yield a crystalline precipitate (50 mg). The crude precipitate was recrystallized from EtOH to give a triol (**9**) as fine needles (33 mg), mp 254—256 °C; $[\alpha]_D^{20}$ +2° (*c* 0.11, $CHCl_3$); IR (Nujol) 3470, 3390, 1735, 1255, and 890 cm^{-1}; ^1H NMR (C_5D_5N) δ 1.14 and 1.24 (3H each, s, 18- and 19-Me), 2.09 and 2.13 (3H each, s, 2Ac), 4.31 (1H, dd, J=8.9 and 1.0 Hz, H_{20a}), 4.40 (1H, dd, J=7.6 and 4.3 Hz, H_6), 4.85 (1H, dd, J=8.9 and 1.4 Hz, H_{20b}), 5.07 (1H, ddd, J=3.0, 2.7, and 1.0 Hz, H_{15}), 5.11 (1H, dd, J=11.3 and 5.6 Hz, H_1), 5.15 (1H, br s, H_{17a}), 5.17 (1H, dd, J=5.4 and 4.6 Hz, H_{11}), 5.48 (1H, br s, H_{17b}), 6.65 (1H, d, J=3.0 Hz, 15-OH), 8.03 (1H, s, 7-OH), and 8.26 (1H, d, J=4.3 Hz, 6-OH); MS *m/e* 450 (M^+), 432 (M^+-H_2O), 390 (M^+-AcOH), 372, and 330 ($M^+-2AcOH$). Found: C, 63.62; H, 7.47%. Calcd for $C_{24}H_{34}O_8$: C, 63.98; H, 7.61%.

Reduction of **2** *with* $NaBH_4$. To a solution of **2** (100 mg) in MeOH (4 ml) and THF (4 ml) were added excess amounts of $NaBH_4$ (40 mg) with ice cooling. The mixture was stirred for 8 h at 0 °C. The solution was then concentrated, diluted with water (10 ml), and extracted with ethyl acetate. The organic layer was washed with water, dried over Na_2SO_4, and evaporated to dryness. The residue was recrystallized from EtOH to afford a triol (62 mg) which was identified by mixed mp, IR, and ^1H NMR comparison with **9**.

Acetylation of **10**. To a solution of **10** (50 mg) in acetic anhydride (6 ml) was added one drop of BF_3-etherate at −60 °C; the mixture was kept for 3 h below −20 °C, then poured into ice-water and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried over Na_2SO_4, and evaporated to dryness. The residue was crystallized from ether-petroleum ether to yield a pentaacetate (**11**) as colorless prisms (33 mg), mp 182—183 °C; IR (Nujol) 1740, 1720, and 1235 cm^{-1}; ^1H NMR δ 1.94, 1.97, 1.99, 2.03, and 2.23 (3H each, s, 5Ac); MS *m/e* 516 (M^+-AcOH), 474, 456 ($M^+-2AcOH$), 432, 414, 396 ($M^+-3AcOH$), 372, 354, 336 ($M^+-4AcOH$), 312, 294, and 281. Found: C, 62.79; H, 6.92%. Calcd for $C_{30}H_{40}O_{11}$: C, 62.48; H, 6.99%. **11** was also obtained by the treatment of **7** with acetic anhydride and BF_3-etherate in a similar manner to that described.

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