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CLERODANE DITERPENE GLUCOSIDES FROM *TINOSPORA RUMPHII*

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Key Word Index—*Tinospora rumphii*; Menispermaceae; clerodane diterpene glucosides; rumphiosides A–F.

Abstract—Seven new diterpene glucosides of the clerodane type were isolated from the stems of *Tinospora rumphii*. Among the seven, one was isolated as an acetyl derivative. The structures of these compounds were established by the application of various spectroscopic techniques.

INTRODUCTION

Tinospora rumphii Boerl. (synonyms *T. tuberculata* Buemee and *T. crispa* Miers) is a climbing vine with stems rich in warts. It is widely distributed in the Philippines and in some southeast Asian countries. This bitter-tasting plant, locally known in the Philippines as Makabuhai, is used for the treatment of stomach troubles, ulcers and fevers, as a tonic and a febrifuge for malaria and smallpox, as a vulnerary for itches and wounds, and many other purposes [1]. A number of chemical constituents have already been isolated from this plant, e.g. phenolic acid amides [2], a phenolic glucoside [2], *N*-acylaporphine alkaloids [3] and some furanoid diterpenes and furanoid diterpene glucosides of the clerodane type [4–6]. Furanoid diterpenes and furanoid diterpene glucosides were also isolated from plants of the same genus, in particular, from the very closely related species *T. cordifolia* [7–12].

A preliminary screening showed that the methanolic extract of *T. rumphii* significantly stimulated glucose transport activity [13]. This prompted us to undertake an investigation of the constituents of this plant. In this paper, we report on the isolation and structure elucidation of seven new diterpene glucosides of the clerodane type, one being an acetyl derivative, from the stems of *T. rumphii*.

RESULTS AND DISCUSSION

The methanol extract of the dried, ground stems of *T. rumphii* was successively partitioned against ether, 1-butanol and water. The butanol fraction was subjected to repeated silica gel and RP-18 column chromatography and preparative HPLC to afford compound **1**. Attempts to isolate other pure constituents from the butanol extract proved to be futile. Hence, some fractions obtained from this extract were combined, then acetylated with acetic anhydride in pyridine to yield three compounds, **Ac-3**, **Ac-4** and **Ac-6**. In the hope of isolating **Ac-3**, **Ac-4** and **Ac-6** in their natural forms, a second extraction was carried out using the procedure described above to give six compounds, **1**–**4**, **6** and **7**. Compounds **Ac-3** and **Ac-6** were isolated in their natural forms **3** and **6**, respectively. Compound **Ac-4** was relabelled as **5**.

The NMR spectral data for compounds **1**–**7** suggested that they were all diterpene glucosides of the clerodane type. The ^1H and ^{13}C NMR spectroscopic data for these compounds suggested certain common structural features. The spectra showed signals corresponding to two angular methyl groups (C-19 and C-20), a methoxycarbonyl group (C-18) and a trisubstituted double bond (C-3 and C-4). The spectra also showed signals attributable to one glucose moiety. In the ^1H NMR spectra, the coupling constants ($J = 7.3$–7.8 Hz) of the signals due to the anomeric protons of the glucopyranoses indicate the glycosidic linkages to have β-configurations. The ^{13}C NMR spectra, when were coupled with distortionless enhancement of polarization transfer (DEPT) experiments, also showed signals attributable to two quaternary carbons (C-5 and C-9), two tertiary carbons (C-8 and C-10), three secondary carbons (C-1, C-7 and C-11) and two oxygenated methine groups (C-6 and C-12). From a biogenetic point of view, the events leading to the formation of the clerodane type bicyclic diterpenes produce the two angular methyls attached to C-5 and C-9 and the two ring junction methines at C-8 and C-10. Moreover, the methoxycarbonyl group is attached to C-4 [14]. The usefulness of ^{13}C NMR chemical shifts of the angular methyls to distinguish between *cis* and *trans* A/B-ring junctions in clerodane diterpenes has been reported [15]. In the case of the *cis* clerodanes, the C-19 methyl carbon atom resonates in a region lower than δ20

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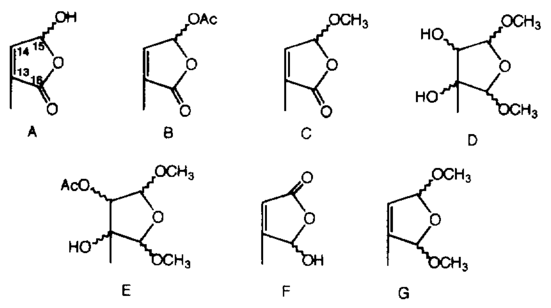
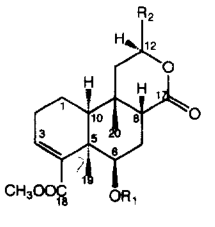
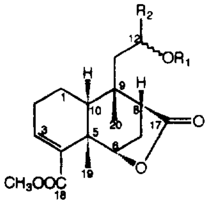




	R_1	R_2
1	β-D-Glc	A
2	β-D-Glc	C
3	β-D-Glc	D
Ac-3	β-D-Glc $(Ac)_4$	E
4	β-D-Glc	D
5	β-D-Glc $(Ac)_4$	G
8 (9)	β-D-Glc $(Ac)_4$	B



	R_1	R_2
6	β-D-Glc	D
Ac-6	β-D-Glc $(Ac)_4$	E
7	β-D-Glc	F

and in the corresponding *trans* compounds, it resonates in the region δ11–19. The C-19 methyl signals of the isolated compounds were found at δ27.2–29.4; thus, their A/B-ring junctions are *cis*. Further structure identification of these compounds involved other 2D NMR analyses.

Compound **1** exhibited a quasi-molecular ion peak, $[M-H]^-$, at *m/z* 567 in its fast atom bombardment mass spectra (FAB MS) measured in the negative ion mode and established that the molecular formula of **1** was $C_{27}H_{36}O_{13}$. In addition to the common structural features presented above, the ^{13}C NMR spectrum of **1** showed a triplet signal at δ24.0 attributable to a secondary carbon (C-2) and a singlet signal at δ173.0 corresponding to an ester carbonyl (C-17). Furthermore, the presence of an α-substituted butenolide attached to C-12 was proposed.

In order to establish the presence of the α-butenolide in **1** in particular, and the compound's structure in general, **1** was acetylated to afford two pentaacetate compounds, **8** and **9**, which are C-15 epimers.

Compounds **8** and **9** had a molecular ion peak at *m/z* 778 in their electron impact mass spectrum (EI MS), corresponding to the molecular formula $C_{37}H_{46}O_{18}$. The NMR spectral data for these compounds (Tables 1 and 2) showed the additional presence of signals attributable to five acetyl groups. The data also showed

Table 1. ^{1}H NMR spectral data for compounds **1**, **2**, **8** and **9** (400 MHz, TMS)

H	**1***	**8**†	**9**†	**2***
1a	1.66 *m*	1.74 *m*	1.82 *m*	1.67 *m*
1b	2.02 *m*	2.10 *m*	2.09 *m*	1.97 *m*
2a	2.17 *m*‡	2.33 *m*§	2.33 *m*§	2.16 *m*‡
2b	2.17 *m*‡	2.33 *m*§	2.33 *m*§	2.16 *m*‡
3	6.74 *t* (3.9)	6.83 *t* (3.9)	6.84 *t* (3.9)	6.74 *t* (3.9)
6	5.15 *br d* (4.4)	4.70 *d* (4.4)	4.71 *d* (4.6)	5.15 *br d* (4.4)
7a	1.91 *m*	1.67 *m*	1.68 *dd* (6.9, 14.4)	1.90 *br dd* (6.3, 13.9)
7b	3.15 *br dd* (3.9, 13.4)	2.73 *ddd* (1.7, 4.6, 13.4)	2.72 *ddd* (1.7, 4.6, 4.4)	3.13 *br dd* (4.4, 13.9)
8	2.34 *m*	2.23 *br d* (5.6)	2.24 *dd* (1.7, 6.9)	2.35 *br d* (6.3)
10	2.57 *d* (6.6)	2.04 *m*	2.04 *m*	2.58 *br d* (5.4)
11a	1.31 *m*	1.20 *m*	1.25 *m*	1.15 *dd* (11.9, 14.5)
11b	2.44 *m*	2.42 *br d* (12.9)	2.43 *m*	2.44 *br d* (14.5)
12	5.51 *br d* (11.7)	5.30 *m*	5.25 *dd* (1.7, 12.2)	5.51 *br d* (11.9)
14	7.34 *m*	7.37 *m*	7.35 *m*	7.56 *m*
15	6.50 *m*	6.94 *m*	6.97 *m*	5.86 *m*
16				
19	1.72 *s*	1.29 *s*	1.28 *s*	1.72 *s*
20	0.96 *s*	1.07 *s*	1.07 *s*	0.96 *s*
CH_3OOC–	3.68 *s*	3.74 *s*	3.74 *s*	3.67 *s*
Glc 1′	5.02 *d* (7.6)	4.60 *d* (7.8)	4.60 *d* (7.7)	5.02 *d* (7.3)
$-OCH_3$				3.45, *s*
$-OCOCH_3$		1.97 *s*	1.96 *s*	
$-OCOCH_3$		2.02 *s*‡	1.99 *s*	
$-OCOCH_3$		2.02 *s*‡	2.01 *s*	
$-OCOCH_3$		2.14 *s*	2.14 *s*	
$-OCOCH_3$		2.18 *s*	2.16 *s*	

*In pyridine-d_5.
†In $CDCl_3$.
‡,§Overlapped signals within a column.
Coupling constant values (Hz) are given in parentheses.

Table 2. ^{13}C NMR chemical shifts of compounds **1–5**, **Ac-3**, **8** and **9** (100 MHz, TMS)

C	**1***	**8**†	**9**†	**2***	**3***	**Ac-3**†	**4***	**5**†
1	16.8 *t*	16.5 *t*	16.6 *t*	16.8 *t*	16.4 *t*	16.1 *t*	16.7 *t*	16.5 *t*
2	24.0 *t*	23.8 *t*	23.8 *t*	24.0 *t*	23.9 *t*	23.8 *t*	24.0 *t*	23.7 *t*
3	141.5 *d*	141.9 *d*	141.9 *d*	141.6 *d*	141.3 *d*	141.7 *d*	141.4 *d*	141.6 *d*
4	136.3 *s*	135.1 *s*	135.1 *s*	136.3 *s*	136.7 *s*	135.4 *s*	136.7 *s*	135.3 *s*
5	40.6 *s*	39.5 *s*	39.5 *s*	40.7 *s*	40.4 *s*	39.5 *s*	40.6 *s*	39.5 *s*
6	78.1 *d*	78.1 *d*	78.0 *d*	78.1 *d*	78.2 *d*	78.0 *d*	78.6 *d*	77.9 *d*
7	29.2 *t*	27.9 *t*	28.0 *t*	29.2 *t*	28.5 *t*	27.7 *t*	28.4 *t*	27.9 *t*
8	46.6 *d*	45.8 *d*	45.9 *d*	46.6 *d*	46.6 *d*	45.8 *d*	46.7 *d*	45.5 *d*
9	35.6 *s*	35.4 *s*	35.5 *s*	35.6 *s*	35.6 *s*	35.0 *s*	35.8 *s*	35.3 *s*
10	36.9 *d*	36.8 *d*	37.0 *d*	37.0 *d*	36.9 *d*	36.2 *d*	36.6 *d*	36.4 *d*
11	40.6 *t*	40.2 *t*	40.5 *t*	40.6 *t*	35.2 *t*	34.7 *t*	35.0 *t*	40.3 *t*
12	70.1 *d*	69.5 *d*	69.6 *d*	70.2 *d*	70.1 *d*	74.9 *d*	69.8 *d*	70.1 *d*
13	136.9 *s*	137.8 *s*	137.8 *s*	138.0 *s*	80.9 *s*	81.1 *s*	81.6 *s*	144.1 *s*
14	146.7 *d*	143.2 *d*	142.8 *d*	144.1 *d*	77.1 *d*	75.7 *d*	77.0 *d*	126.7 *d*
15	99.0 *d*	92.8 *d*	92.6 *d*	103.4 *d*	113.3 *d*	108.0 *d*	112.6 *d*	108.3 *d*
16	169.8 *s*	170.9 *s*	171.0 *s*	169.0 *s*	110.2 *d*	108.6 *d*	109.6 *d*	106.6 *s*
17	173.0 *s*	171.6 *s*	171.8 *s*	173.0 *s*	174.3 *s*	172.1 *s*	174.8 *s*	171.8 *s*
18	167.5 *s*	167.0 *s*	167.1 *s*	167.5 *s*	168.6 *s*	167.1 *s*	167.5 *s*	167.1 *s*
19	27.7 *q*	26.9 *q*	26.9 *q*	27.7 *q*	28.1 *q*	27.3 *q*	29.4 *q*	27.6 *q*
20	27.5 *q*	27.8 *q*	27.8 *q*	27.5 *q*	27.3 *q*	27.0 *q*	27.7 *q*	27.0 *q*
$\underline{\mathrm{C}}\mathrm{H_3OOC}$-	51.5 *q*	51.7 *q*	51.7 *q*	51.5 *q*	51.5 *q*	51.6 *q*	51.4 *q*	51.6 *q*
Glc 1′	105.7 *d*	101.7 *d*	101.5 *d*	105.6 *d*	105.3 *d*	101.6 *d*	105.8 *d*	101.6 *d*
2′	75.4 *d*	71.4 *d*	71.5 *d*	77.1 *d*	77.8 *d*	71.5 *d*	76.7 *d*	71.5 *d*
3′	78.1 *d*	72.9 *d*	72.9 *d*	78.1 *d*	79.1 *d*	73.2 *d*	77.9 *d*	72.9 *d*
4′	70.3 *d*	68.6 *d*	68.6 *d*	75.4 *d*	75.3 *d*	68.6 *d*	75.3 *d*	68.7 *d*
5′	77.0 *d*	71.8 *d*	71.8 *d*	77.9 *d*	78.6 *d*	72.3 *d*	77.9 *d*	71.9 *d*
6′	62.0 *t*	61.3 *t*	61.3 *t*	62.1 *t*	62.2 *t*	61.6 *t*	61.8 *t*	61.4 *t*
$-\mathrm{OCH_3}$				56.7 *q*	54.9 *q*	55.4 *q*	55.4 *q*	52.8 *q*
					55.9 *q*	56.1 *q*	56.0 *q*	54.1 *q*
$-\mathrm{OCOCH_3}$		170.3 *s*	170.3 *s*			170.9 *s*		168.6 *s*
$-\mathrm{OCOCH_3}$		169.4 *s*	169.4 *s*			170.4 *s*		169.3 *s*
$-\mathrm{OCOCH_3}$		169.1 *s*	169.2 *s*			170.2 *s*		170.4 *s*
$-\mathrm{OCOCH_3}$		169.1 *s*	168.8 *s*			169.4 *s*		170.9 *s*
$-\mathrm{OCOCH_3}$		168.4 *s*	168.4 *s*			168.5 *s*		
$-\mathrm{OCOCH_3}$		20.8 *q*	20.8 *q*			21.0 *q*		20.8 *s*
$-\mathrm{OCOCH_3}$		20.7 *q*	20.6 *q*			20.6 *q*		20.7 *q*
$-\mathrm{OCOCH_3}$		20.7 *q*	20.6 *q*			20.6 *q*		20.6 *q*
$-\mathrm{OCOCH_3}$		20.6 *q*	20.6 *q*			20.6 *q*		20.6 *q*
$-\mathrm{OCOCH_3}$		20.6 *q*	20.2 *q*			20.6 *q*		

*In pyridine-d_5.
†In $\mathrm{CDCl_3}$.

two singlet signals at δ_C 137.8 (137.8) and 170.9 (171.0), two doublet signals at δ_C 143.2 (142.8) and 92.8 (92.6) and two multiplet signals at δ_H 7.37 (7.35) and 6.94 (6.97). These signals were attributed to an α-butenolide group, thus establishing the presence of this group in **1**. Moreover, the presence of this group was confirmed from the NMR data for **1** obtained from the second extraction of the plant material.

A shift of a one proton multiplet at δ6.50 in the spectrum of **1** to δ6.94 (6.97) on acetylation and the presence of double signals at δ99.0 and 92.8 (92.6) in the ^{13}C NMR spectra of **1** and **8** (**9**), respectively, indicated the presence of a hydroxyl group at the C-15 position.

The position of the glucose moiety was established through HMBC experiments. Significant correlations were observed between the signals corresponding to C-6 and the anomeric proton, and between the anomeric carbon and H-6, establishing its position to be at C-6. Similarly, the position of the C-17 ester carbonyl was established on the basis of HMBC correlations between H-8 and C-17. Since C-12 is an oxygen-bearing carbon, it was deduced that the ester carbonyl is in fact a δ-lactone ring existing between C-8 and C-12.

The relative configuration of **1** was determined by NOE differential spectral measurements of **1** and its acetyl derivative. When the signal corresponding to H-10 was irradiated, NOEs were observed for the signals corresponding to H-12 and the Me-19 protons, while irradiation of the H-12 signal showed a NOE for the signal of H-10. Irradiation of the signal of the Me-19 protons gave rise to NOEs for the signals of H-10, H-6 and the anomeric proton. On irradiation of the H-6 signal, NOEs were observed for the signals of the Me-19 protons and the anomeric proton, while irradiation of the anomeric proton signal gave rise to NOEs for the signals of H-6 and the Me-19 protons. Furthermore, irradiation of the signals corresponding to the Me-20 protons gave rise to NOEs for the signals corresponding to H-8 and one of the H-7 protons (δ1.91), while irradiation of the H-8 signal showed NOE for the signal of the Me-20 protons. These results, which are summarized in Fig. 1, showed that H-10, Me-19, H-6 and H-12 are α-oriented while Me-20 and H-18 are β-oriented.

From these data, together with the results of ^1H–^1H COSY and HSQC experiments, and a comparison with reported data of closely related compounds, the structure of **1** was determined as shown. This new compound was give the name rumphioside A.

Compound **2** has the molecular formula $C_{28}H_{38}O_{13}$ as determined by negative ion HR FAB mass spectroscopy. The spectroscopic data for **2** were very similar to those observed for **1** except for the following features: (a) the ^1H NMR spectrum contained an additional singlet signal at δ3.45 (Table 1) attibutable to a methoxyl group, (b) the ^{13}C NMR spectrum contained a quartet signal at δ56.7 (Table 2), and (c) the ^{13}C NMR data showed that the dioxygenated carbon (C-15) and C-14 resonances occurred further downfield by 4.4 and 2.6 ppm, respectively. These features were attributed to a methoxyl group attached to C-15. Thus, while **1** has a hydroxybutenolide group, **2** has a methoxybutenolide group. Based on **1**, the structure and relative configuration of **2** were established as shown and the compound was given the name rumphioside B.

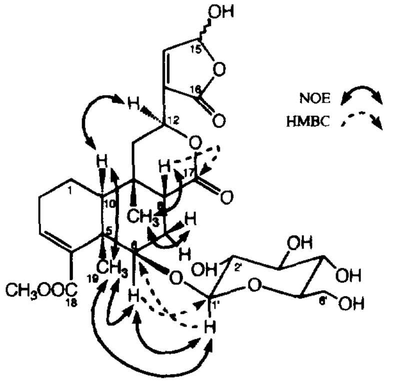
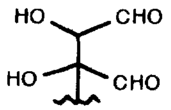


Fig. 1. Important NOEs and ^1H–^{13}C long-range correlations observed for **1**.



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Possible natural form of the group attached to C-12 in 3 and 4

Compound **3** has the molecular formula $C_{29}H_{44}O_{15}$ as revealed by its HR FAB mass spectral data. Aside from the structural features common to all of the isolated compounds, additional features were observed for **3**. Its ^1H NMR spectral data (Table 3) showed two more singlet signals at δ3.38 and δ3.42 corresponding to two methoxyl groups. The presence of these groups was supported by the appearance of two quartet signals at δ54.9 and 55.9 in the ^{13}C NMR spectrum (Table 2). The spectrum also showed one singlet signal at δ80.9 and three doublet signals at δ77.1, 113.3 and 110.2, which were attributed to four oxygenated carbons (C-13 to 16) and a triplet signal at δ23.9 corresponding to a secondary carbon (C-2). From these data and from a comparison with the data for **2**, it was deduced that C-13 and C-14 each has a hydroxyl group, and C-15 and C-16 each has a methoxyl group.

Acetylation of **3** with acetic anhydride in pyridine at room temperature gave **Ac-3**, with a molecular formula of $C_{39}H_{54}O_{20}$, as revealed by its EI mass spectrum. Comparison of the spectral data for **Ac-3** with those for **3** showed additional signals corresponding to five acetyl groups, four of which were attributed to the acetylated sugar moiety and one was attributed to the acetylated hydroxyl group attached to C-14. The hydroxyl group attached to C-13 was not acetylated since it is a tertiary group. These results supported the deduction that hydroxyl groups are attached to C-13 and C-14, and the methoxyl groups are attached to C-15 and C-16. Compound **3** differs from **2** in that, instead of a methoxybutenolide group, it has a tetrahydrofuran ring system attached to C-12. The relative stereochemistry of **3** was determined to be the same as **1** based on a NOESY experiment carried out on **Ac-3**. Based on the above data, the structure of **3** was identified as shown and the compound was given the name rumphioside C.

Compound **4** has the same molecular formula, $C_{29}H_{44}O_{15}$, as that of **3** based on its HR FAB mass spectral (negative) data. Analysis of the spectral data for **4** revealed that it is an epimer of **3**. Thus, **4** was given the name rumphioside C-1.

In the structures of **3** and **4**, it is possible that the tetrahydrofuran ring attached to C-12 is an artifact formed from dialdehyde during the extraction of the plant material with methanol.

Compound **5** showed a molecular ion peak at *m/z* 765 $[M]^+$ in its EI mass spectrum, which corresponded to the molecular formula $C_{37}H_{50}O_{17}$. The ^1H and ^{13}C NMR spectra for **5** (Tables 2 and 3) showed signals at δ_H3.35 and 3.38, and δ_C 52.8 and 54.1 corresponding to two methoxyl groups attached to C-15 and C-16, and signals attributable to four acetyl groups. The ^{13}C NMR spectrum also showed signals at δ144.1 and 23.7, which were assigned to a trisubstituted double bond (C-13 and C-14) and a secondary carbon (C-2), respectively. These signals were in addition to those of the common struc-

Table 3. ^{1}H NMR spectral data for compounds **3–5** and **Ac-3** (400 MHz, TMS)

H	**3***	**Ac-3†**	**5†**	**4***
1a	1.76 *m*	1.67 *m*	1.70 *m*	1.46 *dd* (6.3, 15.6)
1b	1.97 *m*‡	1.95 *m*	1.95 *m*	1.97 *m*§
2a	2.10 *m*§	2.30 *m*‡	2.30 *m*§	2.06 *m*‡
2b	2.10 *m*§	2.30 *m*‡	2.30 *m*§	2.06 *m*‡
3	6.68 *t*	6.77 *t* (3.9)	6.78 *t* (3.9)	6.70 *t* (3.9)
6	4.81 *br d*	4.66 *br d* (3.4)	4.65 *br d* (4.1)	4.51 *br d* (3.5)
7a	1.97 *m*‡	1.65 *m*	1.62 *m*	1.88 *br dd* (7.0, 13.9)
7b	3.14 *dd*	2.65 *ddd* (2.2, 3.4, 14.2)	2.71 *br dd* (1.9, 14.0)	3.12 *ddd* (1.5, 3.5, 13.9)
8	2.27 *br d*	2.10 *dd* (2.2, 6.5)	2.16 *dd* (1.9, 6.6)	2.31 *dd* (1.5, 7.0)
10	2.53 *m*	1.99 *m*	1.98 *m*	2.57 *br d* (6.6)
11a	1.86 *dd*	1.54 *dd* (12.6, 14.4)	1.38 *d* (6.6, 14.2)	1.97 *m*§
11b	2.10 *m*§	2.02 *m*	2.05 *dd* (12.5, 14.2)	2.47 *br d* (14.8)
12	5.12 *br d*	4.78 *dd* (3.0, 12.4)	5.09 *m*	
14	5.32 *d*	5.42 *d* (4.2)	6.15 *br s*	5.49 *br d* (1.7)
15	5.22 *br d*	5.12 *d* (4.2)	5.89 *dd* (3.9, 14.6)	5.45 *br d* (3.9)
16	5.17 *br s*	4.98, *br s*	5.13 *m*	5.41 *br s*
19	1.74 *s*	1.24 *s*	1.24 *s*	1.68 *s*
20	0.99 *s*	1.02 *s*	1.03 *s*	0.92 *s*
CH_3OOC–	3.65 *s*	3.72 *s*	3.71 *s*	3.66 *s*
–OCH_3	3.38 *s*	3.47 *s*	3.35 *s*	3.49 *s*
–OCH_3	3.42 *s*	3.49 *s*	3.38 *s*	3.63 *s*
Glc 1′	4.97 *d*	4.63 *d* (7.8)	4.56 *d* (7.8)	4.92 *d* (7.8)
–$OCOCH_3$		1.98 *s*	1.95 *s*	
–$OCOCH_3$		2.01 *s*	1.97 *s*	
–$OCOCH_3$		2.02 *s*	1.99 *s*	
–$OCOCH_3$		2.11 *s*	2.11 *s*	
–$OCOCH_3$		2.15 *s*		

*In pyridine-d_5.

†$CDCl_3$.

‡,§Overlapped signals within a column.

Coupling constant values (Hz) are given in parentheses.

tural features of the compounds discussed earlier. The relative configuration of **5**, which is the same as that of the compounds already discussed, was determined by NOESY experiments. From these data, the structure of **5** was identified as shown and the compound was given the name rumphioside Ac-D.

Compound **6** is an isomer of **3** with the molecular formula $C_{29}H_{44}O_{15}$ as revealed by its negative HR FAB mass spectral data. Analysis of the ^{1}H and ^{13}C NMR spectra of **6** (Table 4) showed, in addition to the common structural features the presence of a substituted tetrahydrofuran ring attached to C-12 as in **3**, an ester carbonyl observed at δ_C 178.9 and a secondary carbon (C-2) at δ_C 24.4.

The positions of the ester carbonyl and the glucose moiety were determined by means of the HMBC experiment. For the ester carbonyl, significant correlations were observed between the signals corresponding to H-6 and the ester carbonyl carbon (C-17) and between H-8 and C-17. This result showed that **6** possesses a γ-lactone ring instead of a δ-lactone ring. The glucose moiety was placed at C-12 based on correlations between the anomeric carbon and H-12, and the anomeric proton and C-12.

The relative stereochemistry at various centres in **6** was established by NOESY experiments. Cross peaks were observed between the signals corresponding to (i) H-10 and Me-19, (ii) H-6 and Me-19, (iii) Me-20 and one of H-7 (δ2.07), (iv) Me-20 and H-8, and (v) H-8 and H-12 (Fig. 2). From these results, it was deduced that H-10, Me-19 and the lactone ring are α-oriented while Me-20, H-8 and H-12 are β-oriented.

Based on these data, in conjunction with ^{1}H–^{1}H COSY and HSQC experiments, the structure of **6** was established and the compound was given the name rumphioside E. The structure was confirmed from analysis of the spectroscopic data of its tetraacetatyl derivative, **Ac-6**.

Compound **7** has the molecular formula $C_{27}H_{36}O_{13}$ based on its HR FAB mass spectrum (negative) data. The ^{1}H and ^{13}C NMR spectral data for **7** (Table 4) showed additional signals corresponding to a secondary carbon, two ester carbonyls and a trisubstituted double bond. Analysis of these data and comparison with those of the compounds already discussed showed that **7** had a γ-lactone ring attached between C-6 and C-8, and a glucose moiety and a β-substituted γ-hydroxybutenolide attached to C-12. Compound **7** differs from **6** in the substituent attached to C-12: **6** has a substituted hydrofuran ring. In addition, while **7** has a β-substituted γ-hydroxybutenolide, **1** has an α-substituted γ-hydroxybutenolide. The relative configuration of **7** was deduced to be the same as that of **6**. The structure of **7** is as

Table 4. ^{1}H and ^{13}C NMR spectral data for compounds **6**, **7** and **Ac-6** (400 and 100 MHz, respectively, TMS)

	6* (^{13}C)	**6*** (^{1}H)	**Ac-6†** (^{13}C)	**Ac-6†** (^{1}H)	**7*** (^{13}C)	**7*** (^{1}H)
1	16.6 *t*	1.60 *m* 1.73 *br dd* (8.2, 14.6)	16.3 *t*	1.86 *m*‡ 1.86 *m*‡	16.6 *t*	1.44 *m*‡ 1.73 *m*
2	24.4 *t*	1.99 *ddd* (4.4, 8.1, 20.9) 2.13 *m*	24.1 *t*	2.40 *m* 2.79 *m*	24.2 *t*	1.80 *m* 2.11 *m*
3	142.6 *d*	6.84 *t* (3.6)	142.4 *d*	6.99 *t* (3.9)	142.7 *d*	6.87 *t* (3.4)
4	134.7 *s*		134.1 *s*		135.0 *s*	
5	39.6 *s*		39.3 *s*		39.5 *s*	
6	82.8 *d*	5.54 *d* (6.1)	83.0 *d*	5.45 *br d* (5.9)	82.9 *d*	5.59 *br d* (5.9)
7	30.0 *t*	2.07 *m* 2.22 *m*	29.3 *t*	1.91 *m* 2.09 *m*	29.5 *t*	1.94 *m* 2.19 *m*
8	46.0 *d*	3.79 *d* (5.6)	45.8 *d*	2.94 *br d* (5.6)	45.3 *d*	3.14 *br d* (5.6)
9	39.7 *s*		38.6 *s*		39.9 *s*	
10	47.0 *d*	1.54 *br d* (6.4)	46.7 *d*	1.36 *m*	47.0 *d*	1.44 *m*‡
11	40.5 *t*	2.31 *br d* (15.1) 2.60 *dd* (11.2, 15.1)	40.5 *t*	1.42 *br d* (13.9) 2.06 *dd* (9.7, 13.9)	44.0 *t*	1.87 *br d* (12.7) 2.01 *m*
12	76.4 *d*	5.50 *m*	74.8 *d*	4.51 *br d* (8.5)	70.7 *d*	5.71 *br d* (9.5)
13	82.0 *s*		83.0 *s*		171.7 *s*	
14	77.2 *d*	5.02 *d* (4.2)	76.9 *d*	5.41 *br d* (2.0)	118.8 *d*	7.05 *br s*
15	112.6 *d*	5.46 *d* (4.2)	108.6 *d*	5.04 *br d* (2.0)	172.0 *s*	
16	109.4 *d*	5.70 *s*	108.1 *d*	4.79 *br d* (2.0)	98.8 *d*	7.19 *br s*
17	178.9 *s*		179.0 *s*		178.2 *s*	
18	167.0 *s*		166.7 *s*		166.8 *s*	
19	27.2 *d*	1.19 *s*	27.3 *q*	1.32 *s*	27.2 *q*	1.36 *s*
20	21.7 *q*	1.48 *s*	20.6 *q*	1.21 *s*	21.1 *q*	1.38 *s*
CH_3OOC–	51.5 *q*	3.64 *s*	51.7 *q*	3.73 *s*	51.6 *q*	3.68 *s*
–OCH_3	53.7 *q*	3.44 *s*‡	54.4 *q*	3.41 *s*		
–OCH_3	55.7 *q*	3.44 *s*‡	56.0 *q*	3.45 *s*		
Glc 1′	102.8 *d*	5.26 *d* (7.8)	99.4 *d*	4.81 *d* (8.0)	102.4 *d*	4.91 *d* (7.3)
2′	75.6 *d*		71.7 *d*		75.3 *d*	
3′	78.8 *d*		72.9 *d*		78.4 *d*	
4′	72.5 *d*		68.7 *d*		71.8 *d*	
5′	78.5 *d*		71.9 *d*		78.2 *d*	
6′	63.3 *t*		62.1 *t*		62.8 *t*	
–$OOCCH_3$			170.5 *s*			
–$OOCCH_3$			170.1 *s*			
–$OOCCH_3$			169.6 *s*			
–$OOCCH_3$			169.6 *s*			
–$OOCCH_3$			161.1 *s*			
–$OOCCH_3$			20.9 *q*	1.99 *s*		
–$OOCCH_3$			20.7 *q*	2.03 *s*		
–$OOCCH_3$			20.6 *q*	2.11 *s*§		
–$OOCCH_3$			20.6 *q*	2.11 *s*§		
–$OOCCH_3$			20.6 *q*	2.12 *s*		

*In pyridine-d_5.

†In $CDCl_3$.

‡,§Overlapped signals within a column.

Coupling constant values (Hz) are given in parentheses.

shown and the compound was given the name rumphioside F.

EXPERIMENTAL

Mp: uncorr.; NMR: TMS as int. standard; HPLC: D-ODS-5 column (20.0 mm i.d. × 25 cm) with differential refractometer as detector; CC: Kieselgel 60 (70–230 mesh, Merck) and LiChroprep RP-18 (Merck); TLC: Silica gel 60 precoated plates, F_{254} (Merck); HPTLC: RP-18 precoated plates, F_{254} (Merck).

Plant material. Tinospora rumphii Boerl. was collected in Los Baños, Laguna, Philippines. The authentication was done by Prof. Juan V. Pancho of the Department of Botany, Institute of Biological Sciences, University of the Philippines at Los Baños (UPLB). Voucher specimens are deposited at the Organic Chemistry and Natural Products

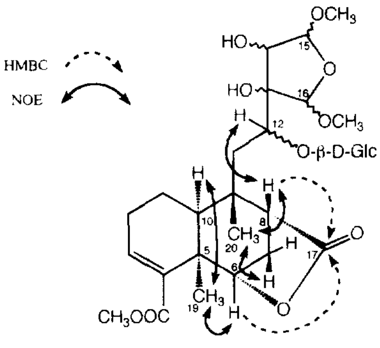

Fig. 2. Important NOEs and ^{1}H–^{13}C long-range correlations observed for **6**.

Research Laboratory, Institute of Chemistry, UPLB, and at the Department of Medicinal Chemistry of Natural Products, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Hiroshima, Japan.

Extraction and isolation of diterpene glucosides. First Extraction. The air dried, ground stem (450 g) of *T. rumphii* was extracted with MeOH at room temp. The MeOH extract was dissolved in H_2O and the soln was successively partitioned against Et_2O and 1-BuOH. The BuOH fr. was subjected to silica gel CC with CH_2Cl_2–MeOH–H_2O (50:8:1 and 50:16:1) to give six frs. Fr. 2 was again subjected to CC on silica gel using the same solvent systems to give six frs. Fr 2–4 afforded **1** (30 mg) after subjecting it to HPLC with 55% MeOH. Fr 2–3 was acetylated.

Rumphioside A (**1**). Powder, $[\alpha]_D^{22}$ $-48.8°$ (MeOH; *c* 0.86). FAB-MS (negative) *m/z*: 567 $[C_{27}H_{36}O_{13}-H]^-$ ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Acetylation of **1**. Compound **1** (12 mg) was dissolved in 4 ml Ac_2O–dry pyridine (1:1) and left overnight at room temp. Solvents were removed by evapn under red. pres. and the residue was purified by HPLC using 65% MeOH to give two pentaacetate derivatives, **8** (2.3 mg) and **9** (2.6 mg).

Compound **8**. Powder, $[\alpha]_D^{21}$ $-34.5°$ (MeOH; *c* 0.09). ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Compound **9**. Powder, $[\alpha]_D^{18}$ $-70.6°$ (MeOH; *c* 0.18). EI-MS (positive) *m/z*: 778 $[C_{37}H_{46}O_{18}]^+$; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Acetylation of Fr. **2**–**3**. Fr. 2–3 (80 mg) was dissolved in 10 ml Ac_2O–dry pyridine (1:1) and left overnight at room temp. The components of the resulting fr. were isolated and purified by HPLC using 65% MeOH and 55% MeCN to give three compounds, **Ac-3**, **Ac-6** and **5**.

Compound **Ac-3**. Powder, $[\alpha]_D^{18}$ $-25.5°$ (MeOH; *c* 0.67). EI-MS (positive) *m/z*: 842 $[C_{39}H_{54}O_{20}]^+$; ^{1}H NMR: Table 3; ^{13}C NMR: Table 2.

Compound **Ac-6**. Powder, $[\alpha]_D^{18}$ $-47.8°$ (MeOH; *c* 0.25). EI-MS (positive) *m/z*: 842 $[C_{39}H_{54}O_{20}]^+$; ^{1}H and ^{13}C NMR: see Table 4.

Rumphioside Ac-D (**5**). White powder, $[\alpha]_D^{19}$ $-66.7°$ (MeOH; *c* 0.15). EI-MS (positive) *m/z*: 766 $[C_{37}H_{50}O_{17}]^+$; ^{1}H NMR: Table 3; ^{13}C NMR: Table 2.

Second extraction. Air-dried powdered stems of *T. rumphii* (1.5 kg) were extracted with MeOH at room temp. After evapn of the solvent, the MeOH extract (125 g) was dissolved in H_2O and the soln was successively partitioned with Et_2O (35 g) and 1-BuOH (50 g). The BuOH extract (20 g) was subjected to silica gel CC with CH_2Cl_2–MeOH–H_2O (20:5:1 and 30:10:1) to give Frs 1–11.

Fr. 2 was further chromatographed over silica gel using stepwise elution with EtOAc–EtOH–H_2O (25:2:1, 20:2:1 and 8:2:1) to give seven frs (Fr. 2-1 to 2-7). Fr. 2-6 gave **2** (21 mg), **3** (18 mg), **4** (13 mg) and **9** (7 mg) and Fr. 2-7 afforded **1** (11 mg), after subjecting these frs to repeated HPLC on ODS, using different proportions of H_2O–MeOH as eluting solvents.

Fr. 5 was sepd into six frs (Fr. 5-1 to 5-6) by silica gel CC using the same solvent system as that used for Fr. 2. From Fr. 5-4, **3** (28 mg) was obtained after subjecting it to RP-18 CC followed by HPLC.

Rumphioside B (**2**). Powder, $[\alpha]_D^{24}$ $-73.7°$ (MeOH; *c* 0.19). HR FAB-MS (negative) *m/z*: 581.2240 $[C_{28}H_{38}O_{13}-H]^-$ requires 581.2234; ^{1}H NMR: Table 1; ^{13}C NMR: Table 2.

Rumphioside C (**3**). Powder, $[\alpha]_D^{22}$ $-0.8°$ (MeOH; *c* 1.22). HR FAB-MS (negative) *m/z*: 631.2589 $[C_{29}H_{44}O_{15}-H]^-$ requires 631.2602; ^{1}H NMR: Table 3; ^{13}C NMR: Table 2.

Rumphioside C-1 (**4**). Powder, $[\alpha]_D^{22}$ $-18.4°$ (MeOH; *c* 0.87). HR FAB-MS (negative) *m/z*: 631.2587 $[C_{29}H_{44}O_{15}-H]^-$ requires 631.2602; ^{1}H NMR: Table 3; ^{13}C NMR: Table 2.

Rumphioside E (**6**). Powder, $[\alpha]_D^{22}$ $-26.7°$ (MeOH; *c* 0.6). HR FAB-MS (negative) *m/z*: 631.2587 $[C_{29}H_{44}O_{15}-H]^-$ requires 631.2602; ^{1}H and ^{13}C NMR: Table 4.

Rumphioside F (**7**). Powder, $[\alpha]_D^{22}$ $-57.1°$ (MeOH; *c* 0.21). HR FAB-MS (negative) *m/z*: 567.2103 $[C_{27}H_{36}O_{13}-H]^-$ requires 567.2077; ^{1}H and ^{13}C NMR: Table 4.

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