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Constituents of *Sindora sumatrana* MIQ. III.[1)] New *trans*-Clerodane Diterpenoids from the Dried Pods

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Seven new *trans*-clerodane-type diterpenoids and three known ones have been identified from the chloroform-soluble fraction of the methanolic extract of *Sindora sumatrana* MIQ. The new compounds, each of which has a β-acetoxy group at the C-7 position, comprise three acid diterpenoids (3, 5, 6) and three neutral ones (8, 9, 10). The remaining one (7) is a dimethyl ester, which was obtained from an acid fraction after methylation with diazomethane. The structures of the isolated compounds were determined by means of spectroscopic methods, mainly two-dimensional NMR techniques, and their relative stereochemistries were determined with the aid of difference nuclear Overhauser effect experiments.

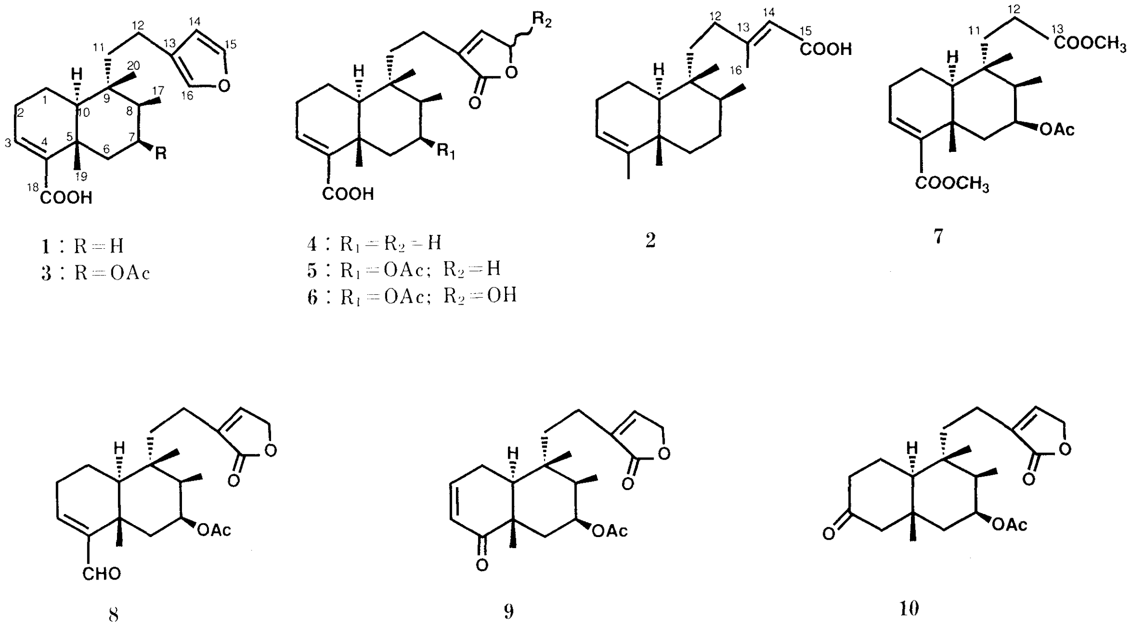
Keywords *Sindora sumatrana*; *trans*-clerodane-type diterpenoid; 7β-acetoxyclerodane; normal-type clerodane; NMR analysis; Leguminosae

The chloroform-soluble fraction of the methanolic extract of the dried pods of *Sindora sumatrana* MIQ. (Leguminosae) was found to be a complex mixture of sesquiterpenoids and both acidic and non-acidic *trans*-clerodane-type diterpenoids. In previous papers,[1,2)] we reported the structure elucidation of the sesquiterpenoids obtained from the neutral part of the chloroform-soluble fraction. We now report the isolation and structure elucidation of ten *trans*-clerodane type diterpenoids.

The chloroform-soluble fraction was partitioned into acidic and neutral parts as described in a previous paper.[2)] Separation of the acidic fraction by a series of column chromatography and preparative TLC procedures gave four new diterpenoids (**3**, **5**, **6**, **7**) and three known ones (**1**, **2**, **4**). On the other hand, separation of the neutral fraction gave three new diterpenoids (**8**, **9**, **10**) along with the sesquiterpenoids reported in previous papers.[1,2)]

Compounds **1**, $[\alpha]_D$ +119° (chloroform), and **4**, $[\alpha]_D$ +109° (chloroform), were both obtained as colorless amorphous solids. They had the molecular formulae $C_{20}H_{28}O_3$ and $C_{20}H_{28}O_4$ [high-resolution MS (HR-MS)], respectively, and showed absorptions due to an α,β-unsaturated carboxyl group in their IR spectra. The ^1H- and ^{13}C-NMR spectra of both **1** and **4** showed the presence of two tertiary (19-CH_3, 20-CH_3) and one secondary (17-CH_3) methyl groups. In addition, the NMR spectra of **1** showed signals which could be ascribed to a β-mono-substituted furan ring,[3)] while those of **4** showed signals due to a butenolide unit.[4,5)]

From these observations and from the analysis of their ^1H–^1H, ^1H–^{13}C and long-range ^1H–^{13}C shift correlation spectroscopy (COSY) spectra, they were determined to be the furanoid clerodane-type diterpenoid, (+)-hardwickiic acid (**1**), and the α-substituted γ-butenolide-containing



1 : R=H
3 : R=OAc

4 : R1=R2=H
5 : R1=OAc; R2=H
6 : R1=OAc; R2=OH

2

7

8

9

10

Chart 1

Table I. 400 MHz ^{1}H-NMR Data[a] for **1**—**10** in $CDCl_3$ (Coupling Constants in Parenthesis)

	1	**3**	**4**	**5**	**6**	**2**	**7**	**9**	**10**	**8**
1	1.50 tdd (13, 12, 6) 1.70 br dd (13, 7)	1.63 tdd (13, 11.5, 7) 1.76 br dd (13, 7)	1.49 dddd (14, 12, 10, 6) 1.70 br dd (14, 7)	1.63 dddd (13, 12, 11, 5.5) 1.78 br dd (13, 7)	1.62 m 1.75 m	1.45 dtd (14, 12, 6) 1.57 br dd (14, 6.5)	1.61 m 1.73 m	2.45 m (2H)	1.84 m 2.03 m	1.59 dddd (13, 12, 10, 6) 1.82 br dd (13, 7)
2	2.19 dddd (20, 13, 7, 3) 2.33 br ddd (20, 6, 4.5)	2.25 dddd (20, 13, 7, 4.5) 2.37 br ddd (20, 7, 3)	2.24 dddd (20, 10, 7, 3) 2.33 dddd (20, 6, 4.5, 1)	2.28 dddd (20, 11, 7, 3) 2.38 br ddd (20, 5.5, 4.5)	2.26 br ddd (20, 10, 7) 2.37 br dt (20, 5)	1.98 m 2.05 m	2.18 dddd (20, 10.5, 7.5, 3) 2.33 dddd (20, 6, 4, 1)	6.97 ddd (10, 5, 3)	2.33 ddd (15, 12, 7) 2.46 ddt (15, 5, 2)	2.42 dddd (20, 12, 7, 3) 2.52 dddd (20, 6, 4.5, 1.5)
3	6.86 dd (4.5, 3)	6.88 dd (4.5, 3)	6.86 dd (4.5, 3)	6.88 dd (4.5, 3)	6.87 br s	5.18 br s	6.60 dd (4, 3)	5.89 dt (10, 2)	—	6.57 dd (4.5, 3)
4	—	—	—	—	—	—	—	—	2.06 dd (13, 2) 2.14 br d (13)	—
6	1.17 td (13, 4.5) 2.44 dt (13, 3)	1.41 dd (15, 3.5) 2.85 dd (15, 3)	1.16 ddd (13, 12, 4.5) 2.44 dt (13, 3)	1.40 dd (15, 3.5) 2.85 dd (15, 3)	1.39 dd (15, 3) 2.84 dd (15, 2.5)	1.18 ddd (13, 10.5, 5) 1.71 dt (13, 2.5)	1.32 dd (15, 3.5) 2.70 dd (15, 3)	1.68 dd (15.5, 3.5) 2.23 dd (15.5, 3)	1.54 dd (15, 3.5) 1.91 dd (15, 3)	1.35 dd (15, 4) 3.04 dd (15, 3)
7	1.42 dtd (13, 4.5, 3) 1.48 qd (13, 3)	5.20 td (3.5, 3)	1.43 dtd (13, 4.5, 3) 1.48 dtd (13, 12, 3)	5.19 td (3.5, 3)	5.17 q (3)	1.43 m (2H)	5.16 td (3.5, 3)	5.24 td (3.5, 3)	5.11 td (3.5, 3)	5.22 td (3.5, 3)
8	1.57 dqd (13, 6.5, 4.5)	1.79 qd (7, 3.5)	1.54 dqd (12, 6.5, 4.5)	1.76 qd (6.5, 3.5)	1.73 qd (7, 3)	1.46 m	1.58 qd (7, 3.5)	1.69 qd (7, 3.5)	1.83 qd (7, 3.5)	1.74 qd (7, 3.5)
10	1.38 br d (12)	1.49 br d (11.5)	1.36 br d (12)	1.47 br d (12)	1.44 br d (12)	1.32 dd (12, 1.5)	1.28 dd (12, 2)	1.93 dd (10, 5.5)	1.80 m	1.45 br d (10)
11	1.56 ddd (14, 12, 4.5) 1.66 ddd (14, 12, 5.5)	1.58 ddd (15, 12, 5) 1.68 ddd (15, 13, 6)	1.50 ddd (14, 13, 4.5) 1.68 ddd (14, 13, 4.5)	1.52 ddd (14.5, 12, 5) 1.68 ddd (14.5, 12, 5)	1.51 ddd (15, 12, 4.5) 1.65 ddd (15, 12, 5)	1.40 td (13, 4.5) 1.53 ddd (13, 12, 5)	1.68 ddd (15, 10.5, 6) 1.76 ddd (15, 11, 6)	1.49 ddd (15, 11, 6) 1.63 ddd (15, 11, 5.5)	1.56 dt (15, 9) 1.67 dt (15, 9)	1.52 ddd (15, 12, 5) 1.68 ddd (15, 12.5, 5)
12	2.18 ddd (14, 12, 5.5) 2.33 ddd (14, 12, 4.5)	2.22 br ddd (13, 12, 6) 2.27 td (13, 5)	2.05 tdt (13, 4.5, 1.5) 2.21 tdt (13, 4.5, 1.5)	2.08 br t (12) 2.15 br t (12)	2.07 m 2.12 m	1.96 ddd (13, 12, 4.5) 2.04 td (13, 5)	2.08 ddd (15, 10.5, 6) 2.14 ddd (15, 11, 6)	2.11 m (2H)	2.22 tq (2H) (9, 1.5)	2.06 m 2.13 m
14	6.25 t (1)	6.25 dd (1.5, 1)	7.11 quintet (1.5)	7.13 t (1.5)	6.87 br s	5.67 q (1)	—	7.11 quintet (1.5)	7.14 quintet (1.5)	7.12 quintet (1.5)
15	7.34 t (1.5)	7.35 t (1.5)	4.77 d (2H) (1.5)	4.79 d (2H) (1.5)	6.11 br s	—	—	4.77 d (2H) (1.5)	4.80 q (2H) (1.5)	4.78 q (2H) (1.5)
16	7.20 br s	7.20 br s	—	—	—	2.17 d (1)	—	—	—	—
17	0.84 d (6.5)	0.93 d (7)	0.83 d (6.5)	0.93 d (6.5)	0.92 d (7)	0.81 d (6)	0.92 d (7)	0.95 d (7)	0.96 d (7)	0.92 d (7)
18	—	—	—	—	—	1.58 s	—	—	—	9.28 s
19	1.26 s	1.45 s	1.26 s	1.44 s	1.42 s	1.00 s	1.44 s	1.25 s	1.07 s	1.36 s
20	0.76 s	1.02 s	0.77 s	1.03 s	1.02 s	0.74 s	1.03 s	1.13 s	1.02 s	1.02 s
$\mathrm{OCOC\underline{H}_3}$	—	2.09 s	—	2.08 s	2.09 s	—	2.06 s	2.06 s	2.05 s	2.07 s
$\mathrm{COOC\underline{H}_3}$	—	—	—	—	—	—	3.67 s, 3.68 s	—	—	—

[a] Assignments are based on the results of ^{1}H–^{1}H and ^{1}H–^{13}C COSY. Assignments of 19- and 20-methyls in **1**—**7** and **9** are based on the results of long-range ^{1}H–^{13}C COSY and in the case of **8** and **10**, by comparison with ^{1}H-NMR data for **5**.

diterpenoid, (+)-3,13-clerodadien-16,15-olid-18-oic acid (**4**), respectively. Compound **1** has been isolated from the Leguminosae plants *Copaifera officinalis*[6] and *C. multijuga*,[7] and also from *Ribes nigrum* (Grossulariaceae).[8] On the other hand, this is the first report of the dextrorotatory form of **4**, although the (−)-*ent*-type ([α]$_D$ −65.9°, chloroform) has been obtained from the Compositae plants *Baccharis patagonica*[9] and *Grangea maderaspatana*.[5]

Compounds **3**, colorless amorphous solid, [α]$_D$ +88° (chloroform), and **5**, [α]$_D$ +87° (chloroform), had the molecular formulae $C_{22}H_{30}O_5$ and $C_{22}H_{30}O_6$, respectively. They both showed a broad absorption band at 3400—2700 cm^{-1} and a strong absorption at 1680—1670 cm^{-1} in their IR spectra. They also exhibited a carbonyl absorption at 1720 cm^{-1}, with **5** showing an extra strong band at 1740 cm^{-1} assignable to a butenolide group. The ^{1}H- and ^{13}C-NMR spectra of **3** and **5** were similar to those of **1** and **4**, respectively, except for a few significant differences. In the ^{1}H-NMR spectra of **3** and **5**, there was an additional signal due to a methyl group (**3**: δ_H 2.09; **5**: δ_H 2.08), which suggested that the two compounds were probably the acetoxy derivatives of **1** and **4**, respectively.

Detailed analysis of the ^{1}H- and ^{13}C-NMR data for **3** and **5** with the aid of ^{1}H–^{1}H and ^{1}H–^{13}C COSY together with long-range ^{1}H–^{13}C COSY established their planar structures as 7-acetoxy-15,16-epoxy-3,13(16),14-clerodatrien-18-oic acid (**3**) and 7-acetoxy-3,13-clerodadien-16,15-olid-18-oic acid (**5**), respectively. Further evidence in support of these structures came from the analysis of the mass spectra of **3** and **5**, which revealed prominent fragment ion peaks at *m/z* 219 (*a*), 201 (*b*) and 173 (*c*) as shown in Chart 2.[10]

Next, a series of difference nuclear Overhauser effect (NOE) experiments carried out on **3** and **5** showed clear NOE's between 19-H_3 and 20-H_3 and between 17-H_3 and 20-H_3, which required a *cis* relationship of the three methyl groups. These results and the fact that irradiation of 10-H did not cause any increase in the intensities of either the 19- or the 20-methyl signals indicated a *trans* stereochemistry of the A and B rings of the decalin system of **3** and **5**. A *trans* A/B ring junction was also evident from

Table II. 100 MHz ^{13}C-NMR Data for **1**—**10** in $CDCl_3$

	1[a)]	**3**[a)]	**4**[a)]	**5**[a)]	**6**[a)]	**2**[a)]	**7**[a)]	**9**[a)]	**10**[b)]	**8**[b)]
1	17.5 t	17.2 t	17.3 t	17.1 t	17.1 t	18.4 t	17.2 t	24.0 t	22.8 t	17.2 t
2	27.5 t	27.3 t	27.4 t	27.2 t	27.3 t	26.9 t	27.0 t	148.5 d	41.9 t	28.4 t
3	140.3 d	140.3 d	140.3 d	140.3 d	140.4 d	120.5 d	136.8 d	127.0 d	210.6 s	151.9 d
4	141.6 s	141.6 s	141.4 s	141.5 s	141.4 s	144.4 s	142.5 s	204.2 s	60.0 t	140.2 s
5	37.6 s	36.8 s	37.5 s	36.8 s	36.7 s	38.2 s	36.8 s	44.4 s	38.0 s	38.2 s
6	35.8 t	39.1 t	35.7 t	39.1 t	39.0 t	36.9 t	39.1 t	36.3 t	44.4 t	38.7 t
7	27.3 t	74.6 d	27.2 t	74.5 d	74.8 d	27.5 t	74.4 d	74.1 d	74.9 d	74.2 d
8	36.3 d	38.1 d	36.2 d	38.1 d	38.1 d	36.4 d	38.3 d	38.0 d	38.9 d	38.2 d
9	38.8 s	38.4 s	38.7 s	38.3 s	38.3 s	38.8 s	38.1 s	38.8 s	38.0 s	39.1 s
10	46.7 d	46.4 d	46.6 d	46.4 d	46.4 d	46.5 d	46.4 d	44.1 d	46.9 d	46.4 d
11	38.7 t	39.5 t	36.0 t	36.9 t	36.8 t	36.4 t	33.9 t	36.2 t	36.9 t	37.0 t
12	18.2 t	18.4 t	19.0 t	19.2 t	19.0 t	35.0 t	28.2 t	19.4 t	19.8 t	19.3 t
13	125.6 s	125.1 s	134.8 s	134.5 s	138.1 s	164.5 s	174.3 s	134.2 s	134.7 s	134.5 s
14	111.0 d	110.9 d	143.6 d	143.8 d	143.5 d	114.9 d	—	144.0 d	144.0 d	143.7 d
15	142.7 d	142.8 d	70.1 t	70.1 t	97.0 d	172.0 s	—	70.1 t	70.6 t	70.2 t
16	138.4 d	138.4 d	174.3 s	174.2 s	172.0 s	19.5 q	—	174.0 s	174.5 s	170.6 s
17	16.0 q	12.0 q	15.8 q	11.9 q	12.0 q	16.0 q	11.9 q	12.1 q	12.4 q	12.0 q
18	172.9 s	172.3 s	172.7 s	172.0 s	171.7 s	18.0 q	167.5 s	—	—	193.6 d
19	20.6 q	22.1 q	20.4 q	22.1 q	22.1 q	20.0 q	22.2 q	19.0 q	21.7 q	21.6 q
20	18.3 q	19.5 q	18.2 q	19.4 q	19.5 q	18.4 q	19.4 q	19.8 q	19.5 q	19.5 q
$\mathrm{OCO\underline{C}H_3}$	—	21.5 q	—	21.4 q	21.5 q	—	21.4 q	21.4 q	21.7 q	21.4 q
$\mathrm{O\underline{C}OCH_3}$	—	170.6 s	—	170.5 s	171.0 s	—	170.5 s	170.1 s	170.8 s	170.6 s
$\mathrm{COO\underline{C}H_3}$	—	—	—	—	—	—	51.7 q 51.3 q	—	—	—

a) Assignments are based on the results of ^{1}H–^{1}H, ^{1}H–^{13}C and long-range ^{1}H–^{13}C COSY. *b*) Assignments are based on the results of ^{1}H–^{1}H and ^{1}H–^{13}C COSY and comparison with the ^{13}C-NMR data for **5**.

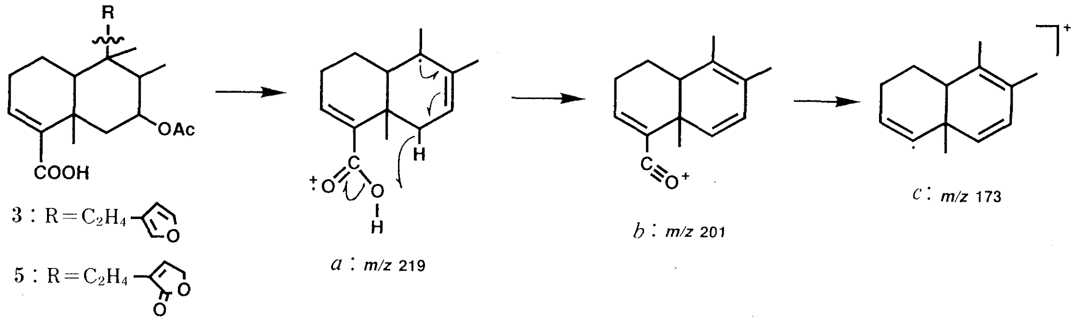


Chart 2

the ^{13}C-NMR chemical shifts of the C-19 methyl carbon (**3**: δ_C 22.1; **5**: δ_C 22.1) and the C-20 methyl carbon (**3**: δ_C 19.5; **5**: δ_C 19.4).[11] With regard to the relative stereochemistry of the 7-acetoxy substituent in **3** and **5**, observation of clear NOE's between the acetoxy methyl group and both 19-H_3 and 20-H_3 indicated a *cis* relationship between it and both tertiary methyl groups, thus defining its stereochemistry as *β*. From the coupling constants of 7-H (**3**: δ_H 5.20, td, $J=3.5$, 3 Hz; **5**: δ_H 5.19, td, $J=3.5$, 3 Hz) and from the inspection of Dreiding models it was clear that the 7-acetoxy group is axial.

From these data, **3** was determined to be (+)-7*β*-acetoxy-15,16-epoxy-3,13(16),14-clerodatrien-18-oic acid and **5** to be (+)-7*β*-acetoxy-3,13-clerodadien-16,15-olid-18-oic acid. These are new compounds, although a compound related to **3**, (+)-7*β*-hydroxyhardwickiic acid, has been isolated from *Copaifera multijuga*.[7]

Compound **6**, a pale yellowish amorphous solid, $[\alpha]_D$ +86° (chloroform), showed the molecular formula $C_{22}H_{30}O_7$, one oxygen more than that of **5**. A comparison of the ^{1}H- and ^{13}C-NMR spectra of **6** with those of **5** (Tables I, II) showed marked similarities between the two compounds and appeared to suggest that the structural difference between them is the presence of an additional hydroxyl group in **6**. The apparent change of the 15-methylene signal at δ_H 4.79 (d, $J=1.5$ Hz) in the ^{1}H-NMR spectrum of **5** to the signal at δ_H 6.11 (1H, br s) in that of **6** clearly indicated that one of the 15-protons in **5** had been substituted by a hydroxyl group in **6**. This was consistent with the downfield shift of the C-15 signal at δ_C 70.1 (t) in the ^{13}C-NMR spectrum of **5** to δ_C 97.0 (d) in that of **6**.

Further analysis of the ^{1}H- and ^{13}C-NMR data of **6**, using ^{1}H–^{1}H and ^{1}H–^{13}C COSY coupled with long-range ^{1}H–^{13}C COSY, confirmed the planar structure of **6** as 7-acetoxy-16-hydroxy-3,13-clerodadien-16,15-olid-18-oic acid. On the basis of the stereochemistry of **5** as well as the results of difference NOE experiments carried out on **6**, together with its ^{1}H-NMR coupling constants, rings A and B of the decalin system in **6** were confirmed to be *trans* and the 7-acetoxy group to be *β* and in an axial orientation. Compound **6** is a new *trans*-clerodane.

Compound **2**, a colorless amorphous solid, $[\alpha]_D$ +26° (chloroform), showed the molecular formula $C_{20}H_{32}O_2$ (HR-MS). The presence of signals due to two tertiary methyl groups (δ_H 0.74 and 1.00) and a secondary methyl group (δ_H 0.81) in the ^{1}H-NMR spectrum of **2** suggested that **2** is also a clerodane-type diterpenoid. In addition, the ^{1}H-NMR spectrum showed two vinyl methyl signals (δ_H 1.58, s; δ_H 2.17, d, J=1 Hz). The signal at δ_H 2.17, which is coupled to a vinylic proton at δ_H 5.67, is characteristic of a β-methyl group in a clerodan-15-oic type α,β-unsaturated acid with the *E*-configuration.[12,13] Detailed analysis of the ^{1}H- and ^{13}C-NMR data of **2** with the aid of ^{1}H–^{1}H and ^{1}H–^{13}C COSY coupled with long-range ^{1}H–^{13}C COSY and the results of a series of difference NOE experiments led us to conclude that **2** is (+)-kolavenic acid. This is the first report of the normal-type kolavenic acid, although the (−)-*ent*-type methyl ester ($[\alpha]_D$ −65.6°, chloroform) has been reported as a constituent of *Hardwickia pinnata* (Leguminosae).[14]

Compound **7**, a colorless amorphous solid, $[\alpha]_D$ +91° (chloroform), was obtained as the dimethyl ester after diazomethane treatment of a chromatographic fraction of the acidic part. The ^{1}H-NMR spectrum was in part similar to those of the previously identified compounds **1**—**6** and showed signals due to two tertiary methyl groups (δ_H 1.03, 20-H$_3$ and 1.44, 19-H$_3$), a secondary methyl group (δ_H 0.92, d, J=7 Hz, 17-H$_3$) and an olefinic proton (δ_H 6.60, dd, J=4, 3 Hz, 3-H) (Table I). In addition the ^{1}H-NMR spectrum of **7** showed signals due to two methoxy groups (δ_H 3.67 and 3.68), an acetoxymethyl (δ_H 2.06) and an oxygenated methine proton (δ_H 5.16, td, J=3.5, 3 Hz, 7-H), suggesting that **7** is a dimethyl ester with an acetoxy substituent. The MS showed a weak ion peak at m/z 380 (M^+) and a fragment ion peak at m/z 320 (M^+ − AcOH). The HR-MS measurement showed the latter ion to have the composition $C_{19}H_{28}O_4$, thus indicating the molecular formula of **7** to be $C_{21}H_{32}O_6$. Since **7** was obtained as a dimethyl ester it was clear that the compound is a nor-clerodane.

Further analysis of the ^{1}H- and ^{13}C-NMR spectra of **7** with the aid of ^{1}H–^{1}H, ^{1}H–^{13}C COSY and long-range ^{1}H–^{13}C COSY led to the planar structure of **7** as dimethyl 7-acetoxy-14,15,16-trinor-3-clerodene-13,18-dioate. As shown for **1**—**6**, the results of a series of difference NOE experiments and the positive optical rotation value of the molecule confirmed **7** to be also a *trans*-clerodane belonging to the normal series. With regard to the C-7 acetoxy group, this was confirmed to be β and in the axial orientation, as in the case of **3**, **5** and **6**. The *ent*-type compound, (−)-nor-hardwickiic acid, has been obtained as the dimethyl ester from *Grangea maderaspatana* (Compositae)[5] and *Eperua leucantha* (Leguminosae)[13] but **7**, the 7β-acetoxy derivative belonging to the normal series, is reported for the first time.

Compounds **9**, $[\alpha]_D$ +29° (chloroform), and **10**, $[\alpha]_D$ +2.4° (chloroform), were isolated as colorless amorphous solids from the neutral part of the chloroform-soluble fraction, and they gave fast-fading color reactions, deep red with Legal's reagent and pink with Kedde's reagent, thus suggesting the presence of α,β-unsaturated γ-butenolide subunit in the two molecules.[15,16] They showed the molecular formulae $C_{21}H_{28}O_5$ and $C_{21}H_{30}O_5$ (HR-MS), respectively, having one carbon less than that of **5**. Their ^{1}H- and ^{13}C-NMR spectra were similar to those of compound **5** (Tables I, II), thus suggesting that **9** and **10** are acetoxy-norclerodane-type diterpenoids possessing an α,β-unsaturated butenolide ring.

The ^{13}C-NMR spectra of **9** and **10** showed signals due to a carbonyl carbon at δ_C 204.2 and δ_C 210.6, respectively, which, in addition to their respective IR absorptions at 1735 and 1720 cm^{-1}, suggested the presence of a ketone group on the decalin ring system. Further analysis of the ^{1}H- and ^{13}C-NMR data with the aid of ^{1}H–^{1}H, ^{1}H–^{13}C and long-range ^{1}H–^{13}C COSY established the structures as 7-acetoxy-4-oxo-18-nor-2,13-clerodadien-16,15-olide (**9**) and 7-acetoxy-3-oxo-18-nor-13-cleroden-16,15-olide (**10**).

With regard to the relative stereochemistry of **9** and **10**, biogenetic considerations as well as examination of the ^{13}C-NMR chemical shifts of their C-19 methyl carbon (**9**: δ_C 19.0; **10**: δ_C 21.7) and C-20 methyl carbon (**9**: δ_C 19.8; **10**: δ_C 19.5),[11] and the coupling constants of 7-H (**9**: δ_H 5.24, td, J=3.5, 3 Hz; **10**: δ_H 5.11, td, J=3.5, 3 Hz) in their ^{1}H-NMR spectra indicated that they were both *trans*-clerodanes with the C-7 acetoxy substituent being axial and thus in the β configuration. These indications were confirmed by difference NOE experiments as in the case of **5**. Both **9** and **10** are new clerodane diterpenoids.

Compound **8**, $[\alpha]_D$ +101° (chloroform), was obtained from the neutral part of the chloroform-soluble fraction as a colorless amorphous solid.[17] Its ^{1}H- and ^{13}C-NMR spectra were found to be similar to those of **5**. There were, however, a few significant differences. In the ^{1}H-NMR spectrum, **8** showed a proton singlet at δ_H 9.28 which suggested the presence of a formyl group in the molecule. This was supported by the ^{13}C-NMR spectrum which showed a carbonyl signal at δ_C 193.6 (d). Following the same procedures as were carried out in the case of **9** and **10**, the compound was finally identified as the *trans*-clerodane diterpenoid, 7β-acetoxy-18-oxo-3,13-clerodadien-16,15-olide, which is being reported for the first time.

The isolation of seven new 7β-acetoxyclerodane-type diterpenoids from *S. sumatrana* appears to offer evidence of a strong tendency of the plant to biosynthesize 7-acetoxy diterpenoids. Perhaps it is also of chemotaxonomic interest that two other Leguminosae plants, *C. multijuga* and *E. leucantha*, which have also yielded 7-hydroxyclerodane derivatives,[7,13] belong to the same Caesalpinioideae subfamily as *S. sumatrana*.[18]

Experimental

Optical rotations were measured on a JASCO DIP-140 digital polarimeter at 26 °C. IR spectra were taken in $CHCl_3$ solutions on a JASCO IR-2 or a Hitachi IR 260-10 spectrophotometer. MS and HR-MS measurements were done on a JEOL D-300 spectrometer using a direct inlet system at the ionization voltage of 70 eV. ^{1}H-, ^{13}C- and two dimensional (2D) NMR and difference NOE spectra were taken with a JEOL JNM-GX400 spectrometer in $CDCl_3$ solutions with tetramethylsilane as an internal standard. Chemical shifts are recorded in δ values and coupling constants in hertz (Hz). Multiplicities of ^{13}C-NMR signals are indicated as s (singlet), d (doublet), t (triplet) and q (quartet).

Column chromatography was carried out over silica gel (Mallinkrodt, 100 mesh) and the eluates were monitored by TLC. Analytical TLC and preparative TLC were carried out on precoated Merck Kieselgel 60 F_{254}

plates (0.25, 0.5, 1.0, 2.0 mm). Detection of separated spots was done by spraying with $Ce(SO_4)_2$–10%H_2SO_4 (1 : 99) reagent. Elution of separated bands was done with MeOH–CH_2Cl_2 (15 : 85) and the eluates were concentrated *in vacuo*.

Separation of Acidic Fraction The acidic fraction (160 g), which was obtained as described in a previous paper,[2] was chromatographed on a silica gel (2 kg) column with $CHCl_3$ (3 l) and MeOH–$CHCl_3$ (5 : 95, 3 l; 10 : 90, 3 l; 20 : 80, 3 l; 30 : 70, 1 l; 50 : 50, 1 l) gradient mixtures. Eluates were collected in 250 ml portions, monitored by TLC and subsequently combined into a total of fifteen fractions [frs.1—4, $CHCl_3$ eluates; frs.5—9, MeOH–$CHCl_3$ (5 : 95) eluates; frs.10—12, MeOH–$CHCl_3$ (10 : 90) eluates; frs.13—14, MeOH–$CHCl_3$ (20 : 80) eluates; fr.15, MeOH–$CHCl_3$ (30 : 70) eluate].

Fractions 2 and 3 were combined (1.1 g) and rechromatographed on a silica gel (60 g) column with $CHCl_3$. The eluates were combined into six fractions (frs. A-1 to A-6). Fractions A-1 to A-3 were combined and subjected to preparative TLC using Et_2O–hexane (60 : 40) to yield compound **1** (330 mg) and a mixture. The mixture was repeatedly separated by preparative TLC using $CHCl_3$ to yield an additional crop of **1** (38 mg) and compound **2** (48 mg).

Fraction 4 (2.2 g) was rechromatographed on a silica gel (60 g) column with $CHCl_3$ and MeOH–$CHCl_3$ (5 : 95) mixture. The eluates obtained were combined into nine fractions (frs. B-1 to B-9). Fraction B-1 [$CHCl_3$ eluate] gave further yield of **1** (35 mg) and frs. B-4 to B-6 [$CHCl_3$ eluates] afforded compound **3** (220 mg).

Fractions 5 and 6 were combined (9.4 g) and rechromatographed over silica gel (300 g) with $CHCl_3$–hexane (50 : 50, 75 : 25) gradient mixtures, $CHCl_3$ and $CHCl_3$–MeOH (5 : 95) mixture. The eluates were combined into a total of thirty-six fractions (frs. C-1 to C-36). Fractions C-3 to C-5 [$CHCl_3$–hexane (50 : 50) eluates] afforded a further yield of **1** (352 mg). Fractions C-20 to C-25 [$CHCl_3$–hexane (75 : 25) eluates] afforded additional **3** (2.1 g). Fractions C-27 to C-30 [$CHCl_3$ eluates] gave compound **4** (1.0 g).

Fraction 7 (15.4 g) was rechromatographed on a silica gel (300 g) column with $CHCl_3$–hexane (50 : 50), $CHCl_3$ and MeOH–$CHCl_3$ (2 : 98, 5 : 95) gradient mixtures. The eluates obtained were combined into twenty-three fractions (frs. D-1 to D-23). Fractions D-10 to D-16 [$CHCl_3$, MeOH-$CHCl_3$ (2 : 98) eluates] yielded compound **5** (926 mg) and fr. D-21 [MeOH–$CHCl_3$ (5 : 95) eluate] afforded compound **6** (140 mg).

Fraction 11 (15 g) was rechromatographed on a silica gel (300 g) column with MeOH–benzene (2 : 98, 5 : 95, 10 : 90, 15 : 85) gradient mixtures. The eluates obtained were combined into fifty-eight fractions (frs. E-1 to E-58). Fractions E-10 to E-16 [MeOH–benzene (5 : 95) eluates] were combined (3 g) and a portion (1 g) was treated with excess CH_2N_2 in Et_2O. Evaporation of the solvent afforded an oily residue (1 g), which was further separated on a silica gel (50 g) column with hexane and acetone–hexane (2.5 : 97.5) mixture to give compound **7** (18 mg).

Isolation of Compounds 8, 9 and 10 Fractions C-20 and C-21 [$CHCl_3$–hexane (50 : 50) eluates], C-44 [$CHCl_3$–hexane (50 : 50, 70 : 30) eluate] and C-47 [$CHCl_3$–hexane (70 : 30) eluate],[2] which were obtained by rechromatography of fraction 8 from the neutral fraction, were used as the starting materials. As described in a previous paper,[2] separation of the combined frs. C-20 and C-21 (105 mg) by preparative TLC gave 8*R*,9*R*-dihydroxy-1(12)-caryophyllene (38 mg). At this stage a polar fraction was obtained which was further subjected to preparative TLC using Et_2O–hexane (80 : 20) to give a small amount of compound **8** (6 mg). On the other hand, frs. C-44 (210 mg) and C-47 (120 mg) were also separated by preparative TLC using Et_2O–hexane (80 : 20) to yield compounds **9** (10 mg) and **10** (3 mg), respectively.

Compound 1 [(+)-Hardwickiic Acid] Colorless amorphous solid, $[\alpha]_D$ +119° (c = 1.8, $CHCl_3$). IR ν_{max} cm^{-1}: 3400—2700 (br), 1680, 1620, 1250, 1200, 1015, 875. ^1H- and ^{13}C-NMR: Tables I and II. MS m/z (%): 316 (M^+, 33), 221 (90), 203 (66), 175 (28), 125 (100), 95 (68), 81 (61). HR-MS: Found 316.2008, Calcd for $C_{20}H_{28}O_3$ (M^+) 316.2037.

Compound 2 [(+)-Kolavenic Acid] Colorless amorphous solid, $[\alpha]_D$ +26° (c = 0.61, $CHCl_3$). IR ν_{max} cm^{-1}: 3400—2700 (br), 1680, 1635, 1250. ^1H- and ^{13}C-NMR: Tables I and II. MS m/z (%): 304 (M^+, 24), 289 (20), 205 (100), 191 (63), 136 (63), 108 (77). HR-MS: Found 304.2402, Calcd for $C_{20}H_{32}O_2$ (M^+) 304.2402.

Compound 3 [(+)-7β-Acetoxy-15,16-epoxy-3,13(16),14-clerodatrien-18-oic Acid] Colorless amorphous solid, $[\alpha]_D$ +88° (c = 2.2, $CHCl_3$). IR ν_{max} cm^{-1}: 3400—2700 (br), 1720, 1680, 1250, 1210, 1020, 875. ^1H- and ^{13}C-NMR: Tables I and II. MS m/z (%): 374 (M^+, 2), 356 (6), 314 (M^+ − AcOH, 62), 219 (a, 100), 201 (b, 21), 173 (c, 17), 125 (35), 95 (56), 81 (39). HR-MS: Found 374.2107, Calcd for $C_{22}H_{30}O_5$ (M^+) 374.2127; Found 314.1879, Calcd for $C_{20}H_{26}O_3$ (M^+ − AcOH) 314.1881.

Compound 4 [(+)-3,13-Clerodadien-16,15-olid-18-oic Acid] Colorless amorphous solid, $[\alpha]_D$ +109° (c = 2.0, $CHCl_3$). IR ν_{max} cm^{-1}: 3400—2700 (br), 1745, 1670, 1260, 1200, 1070, 1050. ^1H- and ^{13}C-NMR: Tables I and II. MS m/z (%): 332 (M^+, 2), 314 (100), 221 (12), 203 (50), 175 (62), 125 (68). HR-MS: Found 314.1867, Calcd for $C_{20}H_{26}O_3$ ($M^+ - H_2O$) 314.1881.

Compound 5 [(+)-7β-Acetoxy-3,13-clerodadien-16,15-olid-18-oic Acid] Colorless amorphous solid, $[\alpha]_D$ +87° (c = 5.25, $CHCl_3$). IR ν_{max} cm^{-1}: 3400—2700 (br), 1740, 1720, 1670, 1240, 1200, 1065, 1045. ^1H- and ^{13}C-NMR: Tables I and II. MS m/z (%): 372 ($M^+ - H_2O$, 19), 330 (M^+ − AcOH, 10), 312 (97), 297 (100), 219 (a, 52), 201 (b, 63), 173 (c, 66), 95 (45). HR-MS: Found 372.1916, Calcd for $C_{22}H_{28}O_5$ ($M^+ - H_2O$) 372.1936; Found 312.1760, Calcd for $C_{20}H_{24}O_3$ ($M^+ - H_2O$ − AcOH) 312.1726.

Compound 6 [(+)-7β-Acetoxy-16-hydroxy-3,13-clerodadien-16,15-olid-18-oic Acid] Pale yellowish amorphous solid, $[\alpha]_D$ +86° (c = 2.33, $CHCl_3$). IR ν_{max} cm^{-1}: 3400—2700 (br), 1760, 1720, 1675, 1255, 1015. ^1H- and ^{13}C-NMR: Tables I and II. MS m/z (%): 388 ($M^+ - H_2O$, 17), 328 ($M^+ - H_2O$ − AcOH, 98), 313 (49), 219 (a, 100), 201 (b, 33), 173 (c, 52), 125 (52). HR-MS: Found 388.1889, Calcd for $C_{22}H_{28}O_6$ ($M^+ - H_2O$) 388.1886; Found 328.1673, Calcd for $C_{20}H_{24}O_4$ ($M^+ - H_2O$ − AcOH) 328.1674.

Compound 7 [Dimethyl (+)-7β-Acetoxy-14,15,16-trinor-3-clerodene-13,18-dioate] Colorless amorphous solid, $[\alpha]_D$ +91° (c = 0.55, $CHCl_3$). IR ν_{max} cm^{-1}: 1730, 1680, 1430, 1350, 1225. ^1H- and ^{13}C-NMR: Tables I and II. MS m/z (%): 380 (M^+, 0.9), 348 (M^+ − MeOH, 10), 320 (M^+ − AcOH, 13), 288 (M^+ − AcOH − MeOH, 100), 273 (42), 233 (68), 201 (28), 173 (30). HR-MS: Found 320.1982, Calcd for $C_{19}H_{28}O_4$ (M^+ − AcOH) 320.1987; Found 288.1722, Calcd for $C_{18}H_{24}O_3$ (M^+ − AcOH − MeOH) 288.1725.

Compound 8 [(+)-7β-Acetoxy-18-oxo-3,13-clerodadien-16,15-olide] Colorless amorphous solid, $[\alpha]_D$ +101° (c = 0.29, $CHCl_3$). ^1H- and ^{13}C-NMR: Tables I and II.

Compound 9 [(+)-7β-Acetoxy-4-oxo-18-nor-2,13-clerodadien-16,15-olide] Colorless amorphous solid, $[\alpha]_D$ +29° (c = 0.67, $CHCl_3$). IR ν_{max} cm^{-1}: 1752, 1735, 1670, 1250, 1075, 1055. ^1H- and ^{13}C-NMR: Tables I and II. MS m/z (%): 360 (M^+, 14), 300 (M^+ − AcOH, 54), 285 (55), 245 (100), 189 (69), 171 (65), 121 (51). HR-MS: Found 360.1937, Calcd for $C_{21}H_{28}O_5$ (M^+) 360.1937; Found 300.1725, Calcd for $C_{19}H_{24}O_3$ (M^+ − AcOH) 300.1725.

Compound 10 [(+)-7β-Acetoxy-3-oxo-18-nor-13-cleroden-16,15-olide] Colorless amorphous solid, $[\alpha]_D$ +2.4° (c = 0.2, $CHCl_3$). IR ν_{max} cm^{-1}: 1750, 1720, 1250, 1055. ^1H- and ^{13}C-NMR: Tables I and II. MS m/z (%): 362 (M^+, 33), 302 (M^+ − AcOH, 39), 287 (24), 191 (100), 173 (21). HR-MS: Found 362.2094, Calcd for $C_{21}H_{30}O_5$ (M^+) 362.2093; Found 302.1874, Calcd for $C_{19}H_{26}O_3$ (M^+ − AcOH) 302.1881.

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