

Neoclerodane Diterpenes from *Amoora stellato-squamosa*

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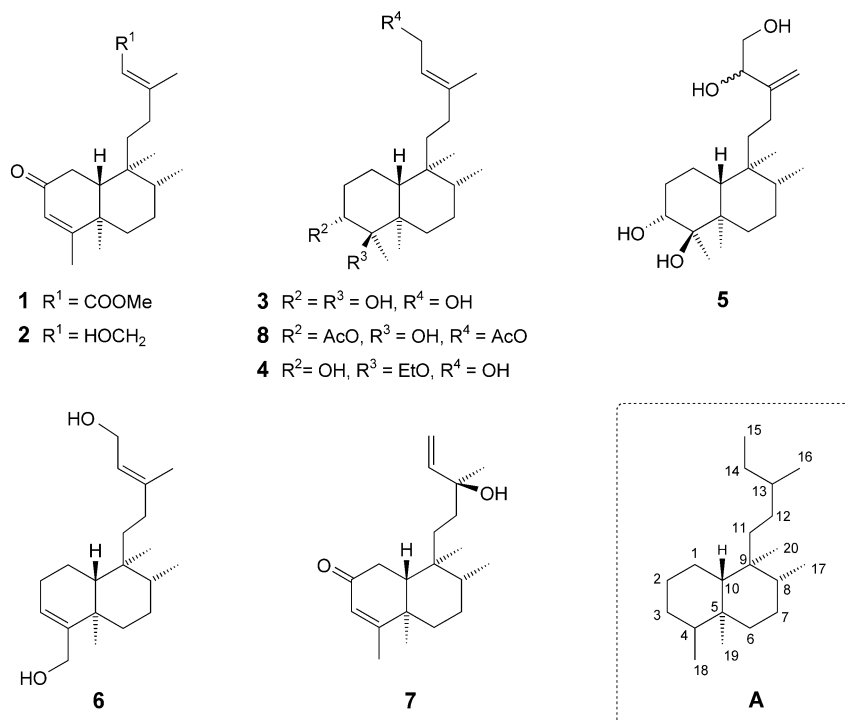
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From the twigs of *Amoora stellato-squamosa*, five new neoclerodane diterpenes have been isolated and characterized, methyl (13*E*)-2-oxoneocleroda-3,13-dien-15-oate (= methyl (2*E*)-3-methyl-5-[(1*S*,2*R*,4*aR*,8*aR*)-1,2,3,4,4*a*,7,8,8*a*-octahydro-1,2,4*a*,5-tetramethyl-7-oxo-naphthalen-1-yl]pent-2-enoate; **1**), (13*E*)-2-oxoneocleroda-3,13-dien-15-ol (= (4*aR*,7*R*,8*S*,8*aR*)-1,2,4*a*,5,6,7,8,8*a*-octahydro-8-[(*E*)-5-hydroxy-3-methylpent-3-enyl]-4,4*a*,7,8-tetramethylnaphthalen-2(1*H*)-one; **2**), (3*α*,4*β*,13*E*)-neoclerod-13-ene-3,4,15-triol (= (1*R*,2*R*,4*aR*,5*S*,6*R*,8*aR*)-decahydro-5-[(*E*)-5-hydroxy-3-methylpent-3-enyl]-1,5,6,8*a*-tetramethylnaphthalene-1,2-diol; **3**), (3*α*,4*β*,13*E*)-4-ethoxyneclerod-13-ene-3,15-diol (= (1*R*,2*R*,4*aR*,5*S*,6*R*,8*aR*)-1-ethoxydecahydro-5-[(*E*)-5-hydroxy-3-methylpent-3-enyl]-1,5,6,8*a*-tetramethylnaphthalen-2-ol; **4**), and (3*α*,4*β*,14*RS*)-neoclerod-13(16)-ene-3,4,14,15-tetrol (= (1*R*,2*R*,4*aR*,5*S*,6*R*,8*aR*)-decahydro-5-[3-(1,2-dihydroxyethyl)but-3-enyl]-1,5,6,8*a*-tetramethylnaphthalene-1,2-diol; **5**), together with two known compounds, (13*E*)-neocleroda-3,13-diene-15,18-diol (**6**) and (13*S*)-2-oxoneocleroda-3,14-dien-13-ol (**7**).

Introduction. – Our continuing investigation on the family Meliaceae has resulted in the isolation of a variety of novel tetranortriterpenoids [1]. So far, tetranortriterpenoids or protolimonoids, considered as chemotaxonomic markers and insect antifeedant active compositions of Meliaceae, have not been obtained from *Amoora yunnanensis* (H. L. Li) C. Y. Wu [2]. The genus *Amoora*, mainly distributed in India and Malaysia, comprises *ca.* 25–30 species, of which six have been found in the Yunnan province, China [3]. The taxonomic position of this genus has given rise to some dispute [4][5], thus, we investigated another *Amoora* species, namely *A. stellato-squamosa* (C. Y. Wu) et H. Li, in the hope of finally corroborating the taxonomy of this genus.

A. stellato-squamosa is a bush growing in Xishuangbanna and Xichou County in the Yunnan province, China [3]. This paper describes the isolation and structural elucidation of the new neoclerodane-type diterpenes **1–5**, which have been isolated, together with the known compounds **6** [6] and **7** [7], from the twigs of this plant. This is, as far as we know, the first report of clerodane-diterpene constituents from the genus *Amoora* [2][8]. The structures of the new compounds were determined by spectroscopic analysis, especially NMR.

Results and Discussion. – The AcOEt-soluble fraction of the EtOH extract of the twigs of *A. stellato-squamosa* was repeatedly chromatographed on silica gel and *RP-18* gel to afford the compounds **1–7**, which were shown to belong to the neoclerodane-type diterpenoids. Generally, neoclerodanes possess a basic C₂₀ skeleton of type **A**, including a C₆ ‘side chain’ (either aliphatic or alicyclic) and a trimethylated *trans*-



decalin (= decahydronaphthalene) moiety, with the Me(17) and Me(20) groups always being α -oriented at C(8) and C(9), respectively [9].

Compound **1**, obtained as colorless needles, was determined to have the molecular formula $\text{C}_{21}\text{H}_{32}\text{O}_3$ based on HR-EI-MS (m/z 332.2364 (M^+ ; calc. 332.2351)), corresponding to six degrees of unsaturation. The IR spectrum showed absorption bands at 1712, 1230, and 1154 cm^{-1} (α,β -unsaturated ester) and 1665 cm^{-1} (α,β -unsaturated ketone). By means of ^1H - and ^{13}C -NMR spectra (Tables 1 and 2, resp.), by HMQC, HMBC, and ROESY experiments, as well as by comparison with literature data (NMR data of **7** [7]), compound **1** was established as methyl (13*E*)-2-oxoneocleroda-3,13-dien-15-oate¹).

The ^{13}C -NMR (DEPT) spectrum of **1** (Table 2) indicated the presence of six Me (δ_{C} 15.7, 17.8, 18.3, 19.0, 19.1, 50.8), five CH_2 (δ_{C} 26.8, 34.0, 34.9, 35.5, 35.5), and four CH groups (δ_{C} 36.0, 45.6, 115.1, 125.5), together with six quaternary C-atoms (δ_{C} 38.7, 39.8, 160.4, 167.1, 172.4, 200.0). The ^1H -NMR spectrum showed the signals for two trisubstituted $\text{C}=\text{C}$ bonds at δ_{H} 5.69 (br. s, 1 H) and 5.62 (br. s, 1 H), one MeO group at δ_{H} 3.64 (s, 3 H), and a CH_2 group (δ_{H} 2.36 (dd , $J = 17.6, 13.6\text{ Hz}$, 1 H) and 2.27 (dd , $J = 17.6, 4.4\text{ Hz}$, 1 H)) next to a $\text{C}=\text{O}$ group (Table 1). These spectral data were quite similar to those of (13*S*)-2-oxoneocleroda-3,14-dien-13-ol (**7**) [7], except for the resonances attributable to the side chain. Hence, compound **1** was expected to contain a neoclerodane skeleton, with a $\Delta^{3,4}\text{C}=\text{C}$ bond and a $\text{C}=\text{O}$ group at C(2)²). This was confirmed by the diagnostic

¹) For systematic names, see the *Exper. Part*.

²) The semi-systematic atom numbering of the basic skeleton **A** (see chemical representation) differs from systematic atom numbers (see systematic names in the *Exper. Part*).

Table 1. ^1H -NMR Data of Compounds **1–6**. Solvents: CDCl_3 for **1**, **2**, **4**, and **6**; CD_3OD for **3** and **5**. Chemical shifts δ in ppm, coupling constants J in Hz.

	1	2	3	4	5	6
$\text{CH}_2(1)$	2.36 (<i>dd</i> , $J = 17.6, 3.6$), 2.27 (<i>dd</i> , $J = 17.6, 4.4$)	2.29–2.37 (<i>m</i>)	1.29–1.35 (<i>m</i>), 1.60–1.67 (<i>m</i>)	1.43–1.50 (<i>m</i>), 1.21–1.30 (<i>m</i>)	1.54–1.58 (<i>m</i>), 1.28–1.31 (<i>m</i>)	1.85–1.93 (<i>m</i>)
$\text{CH}_2(2)$	–	–	1.99 (<i>tt</i> -like, $J = 13.7, 4.0$), 1.60–1.67 (<i>m</i>)	1.89 (<i>m</i>), 1.58 (<i>m</i>)	2.0 (<i>tt</i> , $J = 13.7, 4.0$), 1.57–1.64 (<i>m</i>)	1.98–2.04 (<i>m</i>)
H–C(3)	5.69 (<i>br. s</i>)	5.68 (<i>d</i> , $J = 1.2$)	3.46 (<i>t</i> , $J = 2.6$)	3.77 (<i>t</i> , $J = 2.7$)	3.47 (<i>t</i> , $J = 2.6$)	5.16 (<i>br. s</i>)
$\text{CH}_2(6)$	1.78 (<i>m</i>), 1.34 (<i>m</i>)	1.79 (<i>m</i>), 1.31–1.38 (<i>m</i>)	1.60–1.67 (<i>m</i>), 1.29–1.35 (<i>m</i>)	1.75 (<i>m</i>), 1.17 (<i>m</i>)	1.60–1.69 (<i>m</i>), 1.31–1.34 (<i>m</i>)	1.36–1.48 (<i>m</i>), 1.13 (<i>m</i>)
$\text{CH}_2(7)$	1.47 (<i>m</i>)	1.45 (<i>m</i>)	1.42–1.46 (<i>m</i>), 1.29–1.35 (<i>m</i>)	1.22–1.41 (<i>m</i>)	1.46–1.50 (<i>m</i>), 1.34–1.37 (<i>m</i>)	1.45–1.51 (<i>m</i>), 1.34–1.41 (<i>m</i>)
H–C(8)	1.49 (<i>m</i>)	1.48 (<i>m</i>)	1.49 (<i>m</i>)	1.38 (<i>m</i>)	1.47 (<i>m</i>)	1.41 (<i>m</i>)
H–C(10)	1.82 (<i>dd</i> , $J = 13.6, 4.3$)	1.85 (<i>m</i>)	1.82 (<i>br. d</i> , $J = 10.8$)	2.0 (<i>dd</i> , $J = 12.4, 2.2$)	1.83 (<i>br. d</i> , $J = 11.0$)	1.66 (<i>m</i>)
$\text{CH}_2(11)$	1.40–1.50 (<i>m</i>), 1.28–1.39 (<i>m</i>)	1.45–1.52 (<i>m</i>), 1.31–1.38 (<i>m</i>)	1.54 (<i>m</i>), 1.32–1.36 (<i>m</i>)	1.24–1.46 (<i>m</i>)	1.52 (<i>m</i>), 1.38–1.41 (<i>m</i>)	1.34–1.41 (<i>m</i>)
$\text{CH}_2(12)$	1.96 (<i>m</i>), 1.85 (<i>m</i>)	1.84 (<i>m</i>), 1.73 (<i>m</i>)	1.94 (<i>t</i> -like, $J = 8.0$)	1.80–1.88 (<i>m</i>)	1.88–1.95 (<i>m</i>)	1.95–2.0 (<i>m</i>)
H–C(14)	5.62 (<i>br. s</i>)	5.34 (<i>td</i> , $J = 6.8, 1.1$)	5.32 (<i>t</i> , $J = 6.8$)	5.39 (<i>td</i> , $J = 6.9, 1.1$)	4.08 (<i>tt</i> , $J = 7.4, 3.7$)	5.56 (<i>t</i> , $J = 6.9$)
$\text{CH}_2(15)$		4.09 (<i>d</i> , $J = 6.8$)	4.04 (<i>d</i> , $J = 6.8$)	4.11 (<i>d</i> , $J = 6.9$)	3.57 (<i>dd</i> , $J = 11.2, 3.9$), 3.44 (<i>ddd</i> , $J = 11.2, 7.4, 1.5$)	4.14 (<i>d</i> , $J = 6.9$)
Me(16)	2.11 (<i>d</i> , $J = 1.2$)	1.62 (<i>s</i>)	1.64 (<i>s</i>)	1.66 (<i>s</i>)	5.05 (<i>br. s</i>), 4.89 (<i>br. s</i>)	1.55 (<i>d</i> , $J = 1.2$)
Me(17)	0.81 (<i>d</i> , $J = 6.2$)	0.80 (<i>d</i> , $J = 6.8$)	0.77 (<i>d</i> , $J = 6.0$)	0.74 (<i>d</i> , $J = 6.3$)	0.79 (<i>d</i> , $J = 5.9$)	0.77 (<i>d</i> , $J = 6.9$)
Me(18)	1.85 (<i>d</i> , $J = 1.2$)	1.85 (<i>d</i> , $J = 1.1$)	1.16 (<i>s</i>)	1.13 (<i>s</i>)	1.18 (<i>s</i>)	4.11 (<i>s</i>)
Me(19)	1.08 (<i>s</i>)	1.08 (<i>s</i>)	1.09 (<i>s</i>)	1.06 (<i>s</i>)	1.11 (<i>s</i>)	0.96 (<i>s</i>)
Me(20)	0.79 (<i>s</i>)	0.77 (<i>s</i>)	0.72 (<i>s</i>)	0.68 (<i>s</i>)	0.75 (<i>s</i>)	0.69 (<i>s</i>)
Others	3.64 (<i>s</i> , MeO)			3.29, 3.35 (<i>2dq</i> , $J = 11.0$, 6.9, MeCH ₂), 1.08 (<i>t</i> , $J = 6.9$, MeCH ₂)		

Table 2. ^{13}C -NMR Data of Compounds **1**–**6**²). Solvents: CDCl_3 for **1**, **2**, **4**, **6**, and **8**; CD_3OD for **3** and **5**. Chemical shifts δ in ppm.

	1	2	3	8	4	5	6
C(1)	34.9	35.0	17.7	16.7	16.2	17.64/17.69	18.2
C(2)	200.0	200.5	31.0	27.4	30.6	31.1	26.9
C(3)	125.5	125.4	77.0	76.9	71.9	77.0	120.5
C(4)	172.4	172.7	77.4	75.5	79.3	77.4	144.4
C(5)	39.8	39.8	42.6	41.4	42.3	42.6	38.6
C(6)	35.5	35.7	33.6	32.9	31.9	33.6	36.9
C(7)	26.8	26.8	27.9	26.6	26.8	27.9	27.4
C(8)	36.0	35.9	37.4	36.7	36.0	37.4	36.2
C(9)	38.7	38.6	39.7	38.6	38.5	39.8	38.1
C(10)	45.6	45.6	41.8	40.3	39.4	41.8	46.3
C(11)	35.5	35.5	38.5	36.0	37.2	38.97/38.92	36.7
C(12)	34.0	32.2	34.2	32.0	33.1	26.85/26.79	29.0
C(13)	160.4	139.7	140.9	143.1	141.3	151.8	144.7
C(14)	115.1	123.3	124.3	117.8	122.9	76.8	125.8
C(15)	167.1	59.3	59.5	61.4	59.5	66.6	58.3
C(16)	19.1	16.6	16.5	16.8	16.0	110.64/110.68	18.0
C(17)	15.7	15.7	16.4	15.9	16.4	16.5	16.0
C(18)	19.0	19.0	21.2	21.1	14.3	21.2	60.7
C(19)	18.3	18.3	17.9	16.8	17.5	17.9	19.9
C(20)	17.8	17.9	18.9	18.3	18.3	18.9	18.3
Others	50.8 (<i>q</i> , MeO)	–	–	170.1, 171.1 (2 <i>s</i> , MeC=O); 21.0, 21.4 (2 <i>q</i> , 2 MeC=O)	56.9 (<i>t</i> , MeCH ₂); 16.3 (<i>q</i> , MeCH ₂)		

^1H -NMR signals for $\text{CH}_2(1)$ and $\text{H}-\text{C}(10)$, which appeared as *dd* (eq. $\text{H}_\beta-\text{C}(1)$ at $\delta_{\text{H}} 2.27$ (*dd*, $J = 17.6, 4.4$ Hz); ax. $\text{H}_\alpha-\text{C}(1)$ at 2.36 (*dd*, $J = 17.6, 13.6$ Hz); ax. $\text{H}_\beta-\text{C}(10)$ at 1.82 (*dd*, $J = 13.6, 4.3$ Hz, 1 H)), confirming the relative configuration of this part of the molecule.

HMBC Experiments revealed long-range correlations between $\text{CH}_2(1)$ ($\delta_{\text{H}} 2.36, 2.27$ (*2dd*)) and C(2), C(5), and C(10) ($\delta_{\text{C}} 200.0, 39.8, 45.6$, resp.); between $\text{H}-\text{C}(3)$ ($\delta_{\text{H}} 5.69$ (*br. s*)) and C(5) and C(1) ($\delta_{\text{C}} 39.8$ and 34.9 , resp.); between Me(18) ($\delta_{\text{H}} 1.85$ (*d*, $J = 1.2$ Hz)) and C(3), C(4), and C(5) ($\delta_{\text{C}} 125.5, 172.4$, and 39.8 , resp.); between Me(17) ($\delta_{\text{H}} 0.81$ (*d*, $J = 6.2$ Hz)) and C(7), C(8), and C(9) ($\delta_{\text{C}} 26.8, 36.0$, and 38.7 , resp.); as well as between Me(20) ($\delta_{\text{H}} 0.79$ (*s*)) and C(10), C(8), and C(9) ($\delta_{\text{C}} 45.6, 36.0$, and 37.8 , resp.). In the ROESY spectrum, the Me(20) H-atoms ($\delta_{\text{H}} 0.79$ (*s*)) showed NOE correlations with Me(19), Me(17), and $\text{H}_\alpha-\text{C}(1)$ ($\delta_{\text{H}} 1.08$ (*s*), 0.81 (*d*), and 2.36 (*dd*), resp.), which suggested their *cis*-relationship.

The structure of the side chain was determined by IR, ^1H - and ^{13}C -NMR, in combination with HMQC, HMBC, and ROESY experiments. The IR data indicated the presence of an α,β -unsaturated ester group ($1712, 1230, 1154\text{ cm}^{-1}$), corresponding to the signals at $\delta_{\text{C}} 50.8$ (MeO), 167.1 (C(15)), 115.1 (C(14)), and 160.4 (C(13)) in the ^{13}C -NMR spectrum, with the Me(16) group ($\delta_{\text{C}} 19.1$) being located at C(13). These assignments were in accord with the HMBC spectrum, which showed the following long-range correlations: MeO ($\delta_{\text{H}} 3.46$) to C(15) ($\delta_{\text{C}} 167.1$); $\text{H}-\text{C}(14)$ ($\delta_{\text{H}} 5.62$) to C(16), C(15), C(13), and C(12) ($\delta_{\text{C}} 19.1, 167.1, 160.4$, and 34.0 , resp.); Me(16) ($\delta_{\text{H}} 2.11$) to C(15), C(13), and C(12) ($\delta_{\text{C}} 167.1, 160.4$, and 34.0 , resp.). Therefore, it was deduced that the side chain was a 3-methylpent-2-enoate moiety. Thereby, the C=C bond had to be (*E*)-configured according to the chemical shift of the Me(16) group ($\delta_{\text{H}} 2.11$) [10], and due to a NOE correlation between the H-atoms of Me(16) and those of the ester MeO group in the ROESY spectrum.

Compound **2**, obtained as a colorless oily solid, had the molecular formula $\text{C}_{20}\text{H}_{32}\text{O}_2$, as derived by HR-ESI-MS (m/z 305.2486 ($[M+1]^+$; calc. 305.2480)). By analysis of the ^1H - and ^{13}C -NMR spectral data of **2** and comparison with those of **1**, the structure of **2** was derived as (13*E*)-2-oxoneocleroda-3,13-dien-15-ol¹).

The ^1H - and ^{13}C -NMR spectra of **2** and **1** were similar, indicating that these two compounds had the same skeleton, except for their aliphatic side chains, as supported by HMBC and HMQC experiments. The signals at δ_{C} 123.3 (*d*) and 139.7 (*s*) indicated the presence of a trisubstituted C=C bond, attributed to C(14) and C(13). The high-field-shifted signal at δ_{C} 59.3 (*t*) was assigned to the oxygenated CH_2 (15) group next to the side chain C=C bond. Accordingly, the side chain was identified as a 3-methylpent-2-en-1-ol moiety. This was confirmed by an HMBC experiment, in which long-range correlations were observed between H–C(15) at δ_{H} 4.09 (*d*, $J = 6.8$ Hz) and both C(14) and C(13); between H–C(14) at δ_{H} 5.34 (*td*, $J = 6.8, 1.0$ Hz) and C(12), Me(16), and C(15); as well as between the Me(16) H-atoms at δ_{H} 1.62 (*s*) and C(12), C(13), and C(14). The chemical shift of the Me(16) H-atoms (δ_{H} 1.62) indicated that the C(13)=C(14) bond was (*E*)-configured [10].

Compound **3**, obtained as a white powder, had the molecular formula $\text{C}_{20}\text{H}_{36}\text{O}_3$, as derived by HR-ESI-MS (m/z 347.2568 ($[M + \text{Na}]^+$; calc. 347.2562)). Its IR spectrum showed an OH absorption at 3416 cm^{-1} . According to its ^1H -NMR (Table 1) and ^{13}C -NMR (Table 2) spectra, assigned by means of HMQC and HMBC experiments, and by comparison of the spectral data with those of compound **2**, the structure of **3** was established as (3 α ,4 β ,13*E*)-neoclerod-13-ene-3,4,15-triol¹.

The molecular formula of **3** suggested three degrees of unsaturation. The ^1H -NMR spectrum showed the typical signals of five clerodane-diterpene Me groups: a *d* at δ_{H} 0.77 ($J = 6.0$ Hz), and four *s* at δ_{H} 0.72, 1.09, 1.16, 1.64, resp. The signals at δ_{H} 5.32 (*t*, $J = 6.8$ Hz, H–C(14)), 4.04 (*d*, $J = 6.8$ Hz, H–C(15)), and a three-atom *s* at δ_{H} 1.64 (Me(16)) revealed the presence of the same side chain at C(9) as in **2**, as confirmed by ^{13}C -NMR (δ_{C} 140.3 C(13), 124.3 C(14), 59.5 C(15), 16.5 C(16)). The signals at δ_{C} 77.0 (*d*) and 77.4 (*s*) suggested two oxygenated C-atoms (HO–C(3) and HO–C(4), resp.), as supported by an HMBC experiment, which indicated the following long-range correlations: *a*) H_{β} –C(2) and H_{α} –C(2) at δ_{H} 1.60–1.65 (*m*) and 1.99 (*tt*-like), resp., with C(10) at δ_{C} 41.8 and C(3) at 77.0; *b*) H–C(3) at δ_{H} 3.46 (*t*) with C(1), C(5), and C(4) at δ_{C} 17.7, 42.6, and 77.4, resp.; and *c*) Me(18) at δ_{H} 1.16 (*s*) with C(3), C(4), C(5), resp. In the ^1H -NMR spectrum (Table 1), the triplet at δ_{H} 3.46 and its small coupling constant ($J = 2.6$ Hz) indicated an axially α -oriented HO–C(3) group.

We tried to determine the relative configuration of **3** by means of a ROESY experiment, but, except for NOEs between Me(16) and H–C(15), H–C(14) and H–C(12), Me(20) and Me(19), H–C(10) and H_{β} –C(2), as well as H–C(3) and Me(18), no useful information could be obtained. The Me(17), Me(18), Me(19), and Me(20) groups were assumed to be α -orientated on the basis of comparison with compounds **1**, **2**, and other 3,4-diol-type neoclerodanes [11][12]. Acetylation of **3** afforded the diacetate **8**. The ^1H -NMR spectrum (see *Exper. Part*) of the latter showed that H–C(3) was deshielded (shift change from δ_{H} 3.46 (**3**) to 4.74 (**8**; $\Delta\delta = 1.28$), and the ^{13}C -NMR spectrum (Table 2) allowed the assignment of C(3) and C(4).

Compound **4**, obtained as a colorless, oily solid, had the molecular formula $\text{C}_{22}\text{H}_{40}\text{O}_3$, as derived by HR-ESI-MS (m/z 375.2882 ($[M + \text{Na}]^+$; calc. 375.2875)). From the ^1H - and ^{13}C -NMR spectra (Tables 1 and 2, resp.), HMQC, HMBC experiments, and by comparison of the spectral data with those of **3**, the structure of **4** was assigned as (3 α ,4 β ,13*E*)-4-ethoxyneclerod-13-ene-3,15-diol¹³.

The ^{13}C -NMR spectrum of **4** showed 22 signals: six Me, eight CH_2 , and four CH groups, and four quaternary C-atoms. Relative to the ^1H -NMR spectrum of **3**, additional resonances were observed at δ_{H} 3.35, 3.29 (*ddq*, $J = 11.0, 6.9$ Hz each, MeCH_2O) and 1.08 (*t*, $J = 6.9$ Hz, MeCH_2O), corresponding to an EtO group (δ_{C} 59.9 (*t*) and 16.3 (*q*)). This was confirmed by an HMBC experiment, which showed cross-peaks for the diastereotopic CH_2 resonances of the 4-EtO group with C(4) at δ_{C} 79.3 (*s*) and 16.3 (*q*, MeCH_2O); and of the corresponding Me resonance at δ_{H} 1.08 with the CH_2 group at δ_{C} 56.9 (*t*). The relative configuration of **4** was determined by comparison with **3** and on biogenetic grounds (co-occurrence of neoclerodane-type diterpenes in

³) The EtO group of **4**, most probably, is no artifact (despite EtOH being used for extraction): first: similar diterpenes have been found where no EtOH had been used during extraction and purification [13][14], and, second, subjecting **3** to the same isolation conditions (see *Exper. Part*) did not lead to **4**.

the same plant). Regarding the configuration at C(4): the signal at δ_{H} 2.0 (H–C(10)) was shifted to higher field relative to that of **3** (δ_{H} 1.82) due to shielding *Van der Waals* effects of the EtO group, which confirmed that the 4-EtO and 4-OH groups of **4** and **3**, resp., are *cis* to H $_{\beta}$ –C(10), and, thus, β -configured.

Compound **5**, a white powder, had the molecular formula C₂₀H₃₆O₄ (HR-ESI-MS: m/z 363.2513 ([*M* + Na]⁺; calc. 363.2511); EI-MS: m/z 340 (*M*⁺)). However, not 20 but 24 C-atom signals were observed in the ¹³C-NMR spectrum, four ‘couples’ of resonances, each of similar intensity, being very close together: δ_{C} 17.64 and 17.69, 26.79 and 26.85, 38.92 and 38.97, and 110.64 and 110.67 ppm (Table 2). Based on the similarities of the NMR spectra of **5** and **3**, we counted each ‘couple’ as one C-atom, and the structure of **5** was determined as (3 α ,4 β ,14*RS*)-neoclerod-13(16)-ene-3,4,14,15-tetrol¹).

The ¹³C-NMR spectral data of **5** (Table 2) were quite similar to those of **3**, except for the side-chain resonances. The ¹H-NMR spectrum (Table 1) showed the signals of a terminal vinyl group at δ_{H} 5.05 (br. *s*) and 4.89 (br. *s*), corresponding to C(13) at δ_{C} 151.8 (*s*) and C(16) at 110.6 (*t*), resp. The signals at δ_{H} 3.57 (*dd*, *J* = 11.2, 3.9 Hz, 1 H) and 3.44 (*ddd*, *J* = 11.2, 7.4, 1.5 Hz, 1 H) indicated an oxygenated CH₂ group (C(15)). The signals at δ_{H} 4.08 (*tt*, *J* = 7.4, 3.7 Hz, 1 H) established an OH group at a CH group (C(14)), as confirmed by HMBC long-range correlations between CH₂(16) (δ_{H} 5.05, 4.89 (2 br. *s*, 1 H each)) and C(12), C(15), C(14), C(13) (δ_{C} 26.8, 66.6, 76.8, 151.8, resp.); between H–C(14) (δ_{H} 4.08 (*tt*, 1 H)) and C(16), C(13), C(12), C(15) (δ_{C} 110.6, 151.8, 26.8, 66.6, resp.); and between CH₂(15) (δ_{H} 3.57 (*dd*, *J* = 11.2, 3.9 Hz, 1 H) and 3.45 (*ddd*, *J* = 11.2, 7.4, 1.5 Hz, 1 H)) and C(15), C(14), C(13).

The relative configuration of **5** was determined by spectral comparison with **3**. The absolute configuration of **5** was not determined, but the observed ‘peak doubling’ in the ¹³C-NMR spectrum suggested that **5** was a C(14)-epimeric mixture (*ca.* 1:1).

Compound **6** was identified as (13*E*)-neocleroda-3,13-diene-15,18-diol – a reduced, oxygenated derivative of **1**. The compound has been described before [6], but its ¹H-NMR data were not given correctly. Our revised NMR data (Tables 1 and 2) were assigned by HMBC and HMQC experiments, and by comparison with those of similar compounds [15].

Experimental Part

General. Silica gel (200–300 mesh) for column chromatography (CC) and silica-gel GF₂₅₄ for TLC were obtained from Qingdao Marine Chemical Factory (China). M.p.: XRC-1 Apparatus; uncorrected. Optical ratios: Horiba SEAP-300 polarimeter. UV Spectra: UV-210A spectrophotometer, λ_{max} in nm (log ϵ). IR Spectra: Bio-Rad FTS-135 spectrophotometer, KBr pellets; in cm^{–1}. NMR Spectra: Bruker AM-400 or DRX-500 spectrometers; δ in ppm rel. to Me₄Si as internal standard, *J* in Hz. EI-MS: VG Autospec-3000 spectrometer, at 70eV; in m/z (rel. int.).

Plant Material. The twigs of *A. stellato-squamosa* were collected in the Xishuangbanna County, Yunnan Province, P. R. China, in January 2002. The plants were identified by Prof. Jing-Yun Cui, Xishuangbanna Botanical Garden, Academia Sinica, China.

Extraction and Isolation. The air-dried twigs of *A. stellato-squamosa* (9.0 kg) were extracted with EtOH/H₂O 9:1 (3 × 25 l) at r.t. The extract was evaporated *in vacuo* to give a black-brown gum, which was suspended in H₂O and extracted with AcOEt. The AcOEt extract (110 g) was subjected to CC (SiO₂; CHCl₃/acetone 1:0 → 1:1) to afford nine fractions (*Fr.* 1–9), as judged by TLC. *Fr.* 2 (50 g), *Fr.* 3 (27 g), and *Fr.* 4 (10 g) were repeatedly chromatographed on SiO₂ (petroleum ether/AcOEt 49:1 → 1:1, 10:1 → 1:1, and 9:1 → 1:1, resp.), affording **1** (40 mg) from *Fr.* 2; **2** (160 mg), **4** (13 mg), and **7** (28 mg) from *Fr.* 3; and **6** (220 mg) from *Fr.* 4. *Fr.* 5 (4 g) was repeatedly chromatographed (1. SiO₂; petroleum ether/Me₂CO 7:3 → 1:1; 2. RP-18 gel, MeOH/H₂O 1:1 → 1:0) to afford **3** (470 mg) and **5** (94 mg).

Methyl (2*E*)-3-Methyl-5-[(1*S*,2*R*,4*aR*,8*a**R*)-1,2,3,4,4*a*,7,8,8*a*-octahydro-1,2,4*a*,5-tetramethyl-7-oxonaphthalen-1-yl]pent-2-enoate (**1**).** Colorless crystals. M.p. 88–89. $[\alpha]_{\text{D}}^{20} = -38.69$ (*c* = 0.17, CHCl₃). UV (CHCl₃): 247

(2.73). IR: 2951, 2877, 1712, 1665, 1646, 1614, 1437, 1381, 1230, 1154, 1055, 924, 869. ^1H - and ^{13}C -NMR: see *Tables 1* and *2*, resp. EI-MS: 332 (16, M^+), 317 (5), 301 (21), 285 (32), 259 (24), 243 (19), 205 (56), 189 (22), 177 (16), 161 (27), 135 (66), 121 (68), 109 (100), 83 (30), 69 (14), 55 (13). HR-EI-MS: 332.2364 (M^+ , $\text{C}_{21}\text{H}_{32}\text{O}_3^+$; calc. 332.2351).

(4aR,7R,8S,8aR)-1,2,4a,5,6,7,8,8a-Octahydro-8-[(E)-5-hydroxy-3-methylpent-3-enyl]-4,4a,7,8-tetramethylnaphthalen-2(1H)-one (**2**). Colorless, oily solid. $[\alpha]_{\text{D}}^{20} = -27.54$ ($c = 0.35$, CHCl_3). UV (CHCl_3): 246 (2.26). IR: 3432, 2929, 2874, 1669, 1438, 1382, 1327, 1284, 1080, 947, 849. ^1H - and ^{13}C -NMR: see *Tables 1* and *2*, resp. EI-MS: 304 (4, M^+), 289 (3), 276 (3), 271 (4), 259 (10), 243 (7), 205 (33), 189 (20), 175 (10), 161 (19), 149 (20), 135 (53), 121 (100), 109 (40), 95 (27), 81 (22), 69 (12), 55 (17). HR-ESI-MS: 305.2486 ($[M + \text{H}]^+$, $\text{C}_{20}\text{H}_{33}\text{O}_2^+$; calc. 305.2481).

(1R,2R,4aR,5S,6R,8aR)-Decahydro-5-[(E)-5-hydroxy-3-methylpent-3-enyl]-1,5,6,8a-tetramethylnaphthalene-1,2-diol (**3**). White powder. M.p. 98–99. $[\alpha]_{\text{D}}^{20} = -12.63$ ($c = 0.20$, CHCl_3). IR: 3416, 2941, 2875, 1667, 1637, 1454, 1382, 1324, 1308, 1093, 1053, 1014, 978, 917, 865. ^1H - and ^{13}C -NMR: see *Tables 1* and *2*, resp. EI-MS: 324 (1, M^+), 306 (6), 291 (2), 263 (18), 245 (7), 223 (71), 207 (90), 189 (100), 177 (37), 163 (57), 149 (43), 137 (55), 135 (54), 123 (54), 121 (49), 109 (63), 95 (94), 81 (46), 67 (30), 55 (34). HR-ESI-MS: 347.2568 ($[M + \text{Na}]^+$, $\text{C}_{20}\text{H}_{36}\text{NaO}_3^+$; calc. 347.2562).

(E)-5-[(1S,2R,4aR,5R,6R,8aR)-6-Acetoxydecahydro-5-hydroxy-1,2,4a,5-tetramethylnaphthalen-1-yl]-3-methylpent-2-enyl Acetate (**8**). A soln. of **3** (50 mg) in anh. pyridine/ Ac_2O (10 ml each) was left to stand at r.t. for 48 h. After usual work-up, the product was purified by prep. TLC (petroleum ether/acetone 2:1): 38 mg of **8**. Colorless, oily solid. ^1H -NMR (500 MHz, CDCl_3)⁴: 4.74 (*d*, $J = 2.9$, $\text{H}-\text{C}(3)$); 1.79 (*dd*, $J = 12.4$, 2.1, $\text{H}-\text{C}(10)$); 4.57 (*d*, $J = 7.1$, $\text{CH}_2(15)$); 5.33 (*td*, $J = 7.1$, 0.8, $\text{H}-\text{C}(14)$); 2.05, 2.07, 1.70, 1.11, 1.08, 0.73 (6s, 6 Me); 0.79 (*d*, $J = 6.0$, Me(17)). ^{13}C -NMR: see *Table 2*. EI-MS: 348 (5, $[M - \text{AcOH}]^+$), 306 (3), 288 (5), 265 (77), 205 (81), 189 (100), 177 (16), 163 (17), 149 (29), 135 (32), 123 (80), 107 (57), 95 (41), 69 (40), 55 (45).

(1R,2R,4aR,5S,6R,8aR)-1-Ethoxydecahydro-5-[(E)-5-hydroxy-3-methylpent-3-enyl]-1,5,6,8a-tetramethylnaphthalen-2-ol (**4**). Colorless, oily solid. $[\alpha]_{\text{D}}^{25} = -22.04$ ($c = 0.43$, CHCl_3). IR: 3448, 2945, 2870, 1710, 1666, 1455, 1385, 1279, 1107, 1062, 1017, 978, 958, 941, 845. ^1H - and ^{13}C -NMR: see *Tables 1* and *2*, resp. EI-MS: 352 (3, M^+), 350 (14), 334 (92), 306 (6), 288 (6), 253 (51), 237 (39), 219 (7), 207 (62), 189 (61), 177 (12), 163 (28), 149 (53), 137 (81), 123 (73), 115 (100), 95 (82), 81 (69), 73 (70), 55 (61), 43 (51). HR-ESI-MS: 375.2882 ($[M + \text{Na}]^+$, $\text{C}_{22}\text{H}_{40}\text{NaO}_3^+$; calc. 375.2875).

(1R,2R,4aR,5S,6R,8aR)-5-[3-(1,2-Dihydroxyethyl)but-3-enyl]decahydro-1,5,6,8a-tetramethylnaphthalene-1,2-diol (**5**). White powder. M.p. 141–163°. $[\alpha]_{\text{D}}^{25} = -8.15$ ($c = 0.18$, MeOH). IR: 3424, 2943, 2873, 1633, 1452, 1384, 1092, 1051, 1018, 978, 901. ^1H - and ^{13}C -NMR: see *Tables 1* and *2*, resp. EI-MS: 340 (7, M^+), 322 (2), 310 (5), 261 (12), 243 (11), 225 (34), 207 (71), 189 (100), 177 (19), 163 (61), 149 (45), 137 (60), 123 (48), 109 (54), 95 (62), 81 (36), 69 (29), 55 (39). HR-ESI-MS: 363.2513 ($[M + \text{Na}]^+$, $\text{C}_{20}\text{H}_{36}\text{NaO}_4^+$; calc. 363.2511).

(E)-3-Methyl-5-[(1S,2R,4aR,8aR)-1,2,3,4,4a,7,8,8a-octahydro-5-(hydroxymethyl)-1,2,4a-trimethylnaphthalen-1-yl]pent-2-en-1-ol (**6**). Colorless, oily solid. $[\alpha]_{\text{D}}^{30} = -23.49$ ($c = 0.15$, CHCl_3). IR: 3406, 2935, 1721, 1654, 1452, 1382, 1284, 1173, 1075, 1001. ^1H - and ^{13}C -NMR: see *Tables 1* and *2*, resp. EI-MS ($\text{C}_{20}\text{H}_{34}\text{O}_2$): 288 (6, $[M - \text{H}_2\text{O}]^+$), 286 (10), 273 (4), 271 (9), 255 (11), 205 (9), 191 (44), 189 (100), 175 (23), 159 (21), 149 (22), 135 (39), 121 (66), 107 (62), 95 (85), 81 (43), 69 (20), 55 (23).

REFERENCES

- [1] S. H. Qi, L. Chen, D. G. Wu, Y. M. Bao, X. D. Luo, *Tetrahedron* **2003**, 59, 4193; X. D. Luo, S. H. Wu, D. G. Wu, Y. M. Bao, S. H. Qi, *Tetrahedron* **2002**, 58, 7797; X. D. Luo, S. H. Wu, D. G. Wu, Y. M. Bao, S. H. Qi, *Tetrahedron* **2002**, 58, 6691; X. D. Luo, S. H. Wu, Y. M. Bao, D. G. Wu, *Phytochemistry* **2000**, 55, 867.
- [2] X. D. Luo, S. H. Wu, Y. B. Ma, D. G. Wu, *Heterocycles* **2000**, 53, 2795.
- [3] Yunnan Institute of Botany, Chinese Academy of Sciences, 'Flora Yunnanica', Science Press, Beijing, 1977, Vol. 1, p. 233.
- [4] H. K. Airy-shaw, J. K. Willis, 'A Dictionary of the Flowering Plants and Ferns', 8th edn., Cambridge University Press, London, N.Y., 1973, p. 54.
- [5] P. D. Pennington, B. T. Styles, *Blumea* **1975**, 22, 419.
- [6] K. Nabeta, T. Oohata, S. Ohkubo, T. Sato, K. Kato, *Phytochemistry* **1996**, 41, 581.
- [7] M. D. Bomm, J. Zukerman-Schpector, L. M. X. Lopes, *Phytochemistry* **1999**, 50, 455.

⁴) Diagnostic signals only.

- [8] C. D. Daulatabad, S. A. M. Jamkhandi, *Phytochemistry* **1997**, *46*, 155; E. A. Aboutabl, F. S. el-Sakhawy, M. M. Fathy, R. M. A. Megid, *J. Essent. Oil Res.* **2000**, *12*, 635.
- [9] M. Bruno, F. Piozzi, S. Rosselli, *Nat. Prod. Rep.* **2002**, *19*, 357.
- [10] S. Bory, M. Fetizon, P. Laszlo, *Bull. Soc. Chim. Fr.* **1963**, 2310.
- [11] K. M. Meragelman, L. A. Espinar, V. E. Sosa, M. L. Uriburu, J. R. de la Fuente, *Phytochemistry* **1996**, *41*, 499.
- [12] F. Nagashima, H. Tanaka, Y. Kan, S. Huneck, Y. Asakawa, *Phytochemistry* **1995**, *40*, 209.
- [13] G. Delgado, H. Cardenas, G. Peláez, A. R. de Vivar, *J. Nat. Prod.* **1984**, *47*, 1042.
- [14] A. Ortega, F. J. Morales, M. Salmón, *Phytochemistry* **1985**, *24*, 1850.
- [15] M. Kuroyanagi, K. Uchida, A. Ueno, M. Satake, K. Shimomura, *Phytochemistry* **1993**, *34*, 1377.

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