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ROSANE DITERPENES AND BIS-DINORDITERPENES FROM *HUGONIA CASTENEIFOLIA*†

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Key Word Index—*Hugonia casteneifolia*; Linaceae; diterpenes; hugorosenone; 18-hydroxyhugorosenone; hugorosediol; hugonone A; hugonone B.

Abstract—From the root bark of *Hugonia casteneifolia* three new rosane-type diterpenes, hugorosenone (3β-hydroxy-l(10),15-rosadien-2-one), 18-hydroxyhugorosenone (3β,18-dihydroxy-l(10),15-rosadien-2-one), hugorosediol (3β,18-dihydroxy-l(10),15-rosadiene), and the two bis-dinorditerpenes, hugonone A and hugonone B, have been isolated. Structural determination was achieved by spectroscopic methods. Extracts of the root bark exhibited cytotoxic activity in the brine shrimp bioassay, and hugorosenone was demonstrated to be the corresponding active principle.

INTRODUCTION

In the course of our chemical investigation of Tanzanian medicinal plants [2–4] we recently isolated several bioactive and hitherto unknown natural products [5–9]. In continuation of these investigations, we analysed the root bark of *Hugonia casteneifolia* Engl., which is used in East Africa as a remedy against intestinal worms, malaria and other ailments [10].

In East Africa the plant occurs as a shrub or climber in coastal forests of Kenya, Tanzania, and in Zanzibar [11]. So far no phytochemical studies have been reported on this or any other species of the genus *Hugonia*.

The observation that hexane and dichloromethane extracts of the root bark of *H. casteneifolia* exhibit a strong activity in the brine shrimp bioassay [12], prompted us to investigate these extracts phytochemically. This resulted in the isolation of three new rosane-type diterpenes, which we named hugorosenone (**1**), 18-hydroxyhugorosenone (**2**) and hugorosediol (**3**), respectively. In addition the two new bis-dinorditerpenes, hugonone A (**4**) and hugonone B (**5**), were isolated.

RESULTS AND DISCUSSION

Repeated chromatography of the *n*-hexane and dichloromethane extracts of the root bark of *H. casteneifolia* yielded the constituents **1**–**5**.

Compounds **1**–**3** showed spectroscopic properties (MS, ^{1}H NMR, ^{13}C NMR) indicative for diterpenes. Homonuclear COSY, HMQC and HMBC experiments (Fig. 1) enabled assignment of the ^{1}H NMR and of all ^{13}C NMR signals and established the structure of **1**. The relative configuration resulted from NOE measurements (Fig. 2).

The NMR spectra of compounds **2** and **3** (Tables 1 and 2) closely resembled those of compound **1**. However, their ^{1}H NMR exhibited 'only' three singlets for methyl groups, but 'additional' doublets of an AB-system which could be attributed to an oxymethylene. This indicated the transformation of one of the methyl groups in **1** to a hydroxymethyl group in **2** and **3**. Furthermore, the ^{13}C NMR of **3** did not exhibit the resonance of any carbonyl carbon atom in accordance with corresponding alterations in the IR and UV spectra. The location of the hydroxymethyl group and complete proof of the structures of **2** and **3** again came from HMQC and HMBC experiments. The relative configurations were established by NOE measurements.

In addition to the rosane-type diterpenes **1**–**3**, compounds **4** and **5** were isolated from the dichloromethane extract. HRMS determined the molecular

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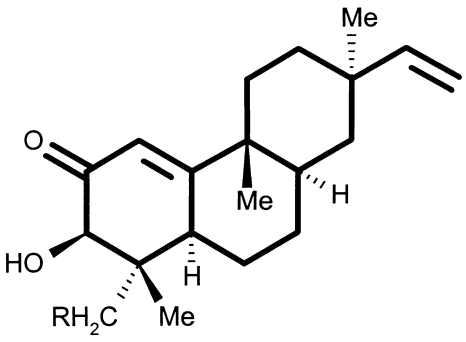
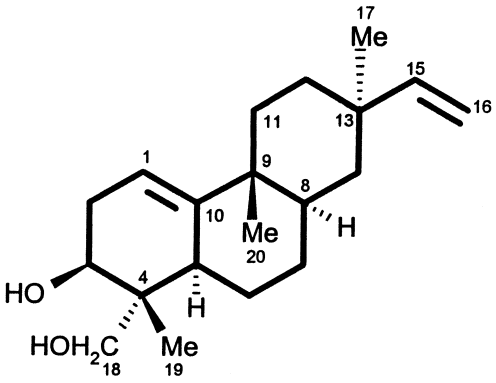
† Part 87 in the series 'Constituents of Tropical Medicinal Plants'. For part 86 see Ref. [1].



1 R = H

2 R = OH



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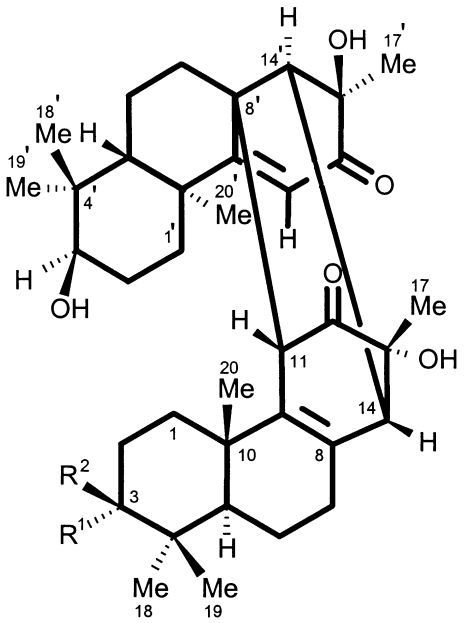


4 R^1 = H, R^2 = OH

5 $R^1 + R^2$ = O

formulae $C_{36}H_{52}O_6$ and $C_{36}H_{50}O_6$ respectively. The structure presented for **4** is the result of extensive homonuclear COSY, HMQC and HMBC measurements (see Fig. 3).

Comparison of the NMR spectra of **4** and **5** indicated closely related structures with the only structural variation at C-3, which carries an α-hydroxy function in **4** but a keto-oxygen in **5**. Structurally, compounds **4** and **5** represent 'dimeric' 15,16-dinorpimaranes, which biogenetically might originate from a Diels-Alder-type cycloaddition of two hypothetical molecules like **6**.

This consideration is supported by the relative configurations established for **4** and **5** from NOE measurements (Fig. 4) and coupling constants, which indicate endo orientation and syn-addition of the two 'monomers'.

Hugonone A (**4**) and B (**5**) are structurally related

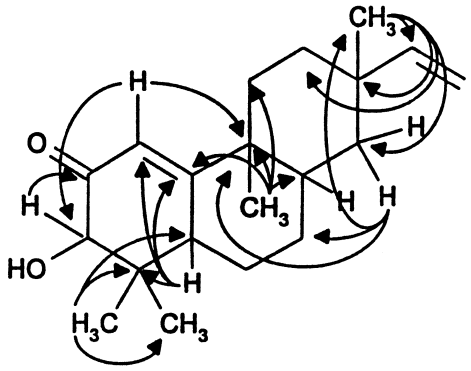


Fig. 1. Important C-H-correlations observed in the HMBC of compound **1**.

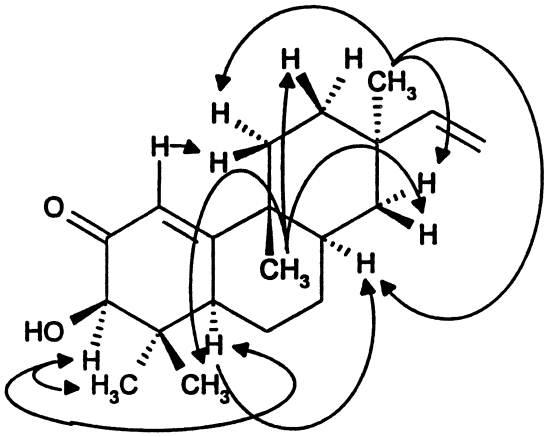


Fig. 2. Important NOEs observed for compound **1**.

Table 1. ^{1}H NMR data of compounds **1**, **2** and **3** (δ in $CDCl_3$, J [Hz])

	1		**2**		**3**	
H	δ	Multiplicity; J	δ	Multiplicity; J	δ	Multiplicity; J
1	6.10	*d*; 3	6.09	*d*; 3	5.40	*ddd*; 5.5, 2.5, 2.5
2a	—	—	—	—	2.28	*
2b	—	—	—	—	1.99	*dddd*; 17.5, 10.5, 4.5, 2.5
3	3.94	*s*	4.34	*s*	3.91	*dd*; 10.5, 6
5	2.62	*ddd*; 13, 5, 3	3.07	*ddd*; 13, 5, 3	2.30	*
6a	1.87	*m*	1.85	*	1.60	*
6b	1.50	*	1.45	*	1.35	*
7a	1.75	*	1.75	*	1.55	*
7b	1.35	*	1.30	*	1.25	*
8	1.75	*	1.85	*	1.75	*dddd*; 12.5, 11, 6.5, 4
11a	1.75	*	1.75	*	1.70	*
11b	1.50	*	1.50	*	1.45	*m*
12a	1.25	*	1.25	*	1.50	*
12b	1.25	*	1.25	*	1.25	*
14a	1.55	*	1.55	*	1.20	*dd*; 13.5, 12.5
14b	1.35	*	1.35	*	1.10	*ddd*; 13.5, 4, 2.5
15	5.81	*dd*; 17.5, 11	5.81	*dd*; 17.5, 11	5.00	*dd*; 17.5, 10.5
16a	4.94	*dd*; 17.5, 1.5	4.94	*dd*; 17.5, 1.5	4.91	*dd*; 17.5, 1.5
16b	4.88	*dd*; 11, 1.5	4.88	*dd*; 11, 1.5	4.84	*dd*; 10.5, 1.5
Me-17	0.99	*s*	0.99	*s*	0.98	*s*
R-CH_2-18†	1.21	*s* (3H)	3.72	*d*; 11	3.75	*d*; 11
			3.58	*d*; 11	3.57	*d*; 11
Me-19	0.65	*s*	0.62	*s*	0.75	*s*
Me-20	1.02	*s*	1.04	*s*	0.92	*s*
-OH	3.75	*br s*	3.83	*br s*	2.40	*br s* (2H)
			2.30	*br s*		

* Signals not resolved; †R = H or -OH

to maytenone (**8**), which has been reported from *Maytenus dispermum* [13, 14]. However, whereas maytenone can be regarded as a Diels-Alder-type cycloaddition product of the abietane derivative **9**, the hugonones represent corresponding products of the dinorpimaradienes **6** and **7**, respectively.

The crude extracts from *Hugonia casteneifolia* exhibited significant activity in the brine shrimp test [12]. This cytotoxic effect was traced in the course of the chromatographic separation procedure, and hugorosenone (**1**) was revealed as the active principle.

EXPERIMENTAL

General

Mps: uncorr. Analytical TLC was performed on precoated plates (silica gel 60 F_{254} Merck) using pet-

Table 2. ^{13}C NMR resonances of compounds **1**, **2** and **3** (δ in $CDCl_3$)

C	1	2	3	C	1	2	3
1	119.9	119.4	115.0	11	34.1	34.2	35.5
2	200.1	200.2	31.8	12	39.6	39.5	32.9
3	80.3	75.7	73.4	13	36.1	36.1	36.3
4	43.6	48.2	40.4	14	32.4	32.4	39.7
5	45.3	38.5	38.1	15	150.4	150.4	151.2
6	17.4	17.1	18.7	16	109.3	109.3	108.7
7	24.8	24.5	25.5	17	22.2	22.2	22.3
8	30.8	30.7	31.4	18	24.3	64.7	68.9
9	38.8	39.0	37.1	19	12.8	9.3	9.2
10	177.2	177.5	149.5	20	18.9	19.0	20.9

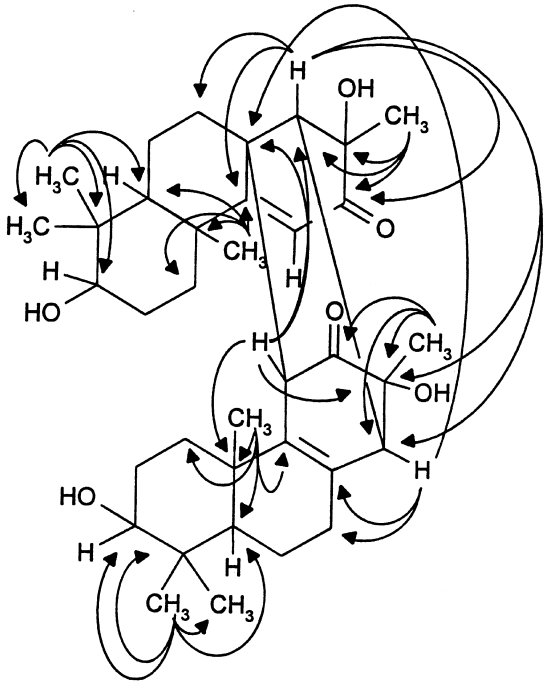


Fig. 3. Important C-H-correlations observed in the HMBC of compound **4**.

rol–Me_2CO (3:2) as the eluant; detection by UV and anisaldehyde reagent [15]. For CC silica gel 60 (Merck) or Sephadex® LH-20 (Pharmacia) were used. Vacuum liquid chromatography (VLC) was performed with silica gel 60 (Merck) using petrol with increasing amounts of EtOAc as eluant. Unless otherwise stated $[\alpha]_D$ in $CHCl_3$ at 21°, UV in MeOH, IR in KBr. ^1H NMR at 360 MHz and ^{13}C NMR at 62.9 MHz in $CDCl_3$ with TMS as int. standard. EIMS at 70 eV with direct inlet; CIMS with CH_4; unless key ions, only ions with rel. intensities $\geqslant 20\%$ and $m/z \geqslant 100$ are presented. The brine shrimp bioassay was performed according to Ref. [12].

Plant material

The root bark of *Hugonia casteneifolia* Engl. was collected from Pugu Forest (about 25 km from Dar es Salaam, Tanzania) in September 1995 and January 1996. The plant was identified by L. B. Mwasumbi and F. M. Mbago from the Herbarium of the University of Dar es Salaam, Department of Botany, where a voucher specimen is preserved under No. FMM 820.

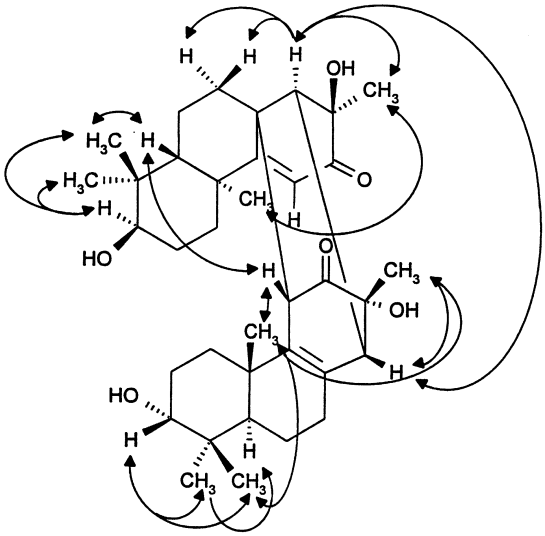


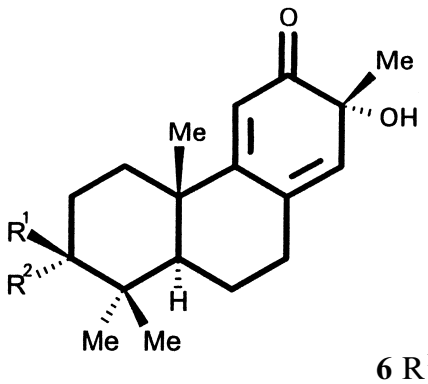
Fig. 4. Important NOEs observed for compound **4**.

Extraction and Isolation

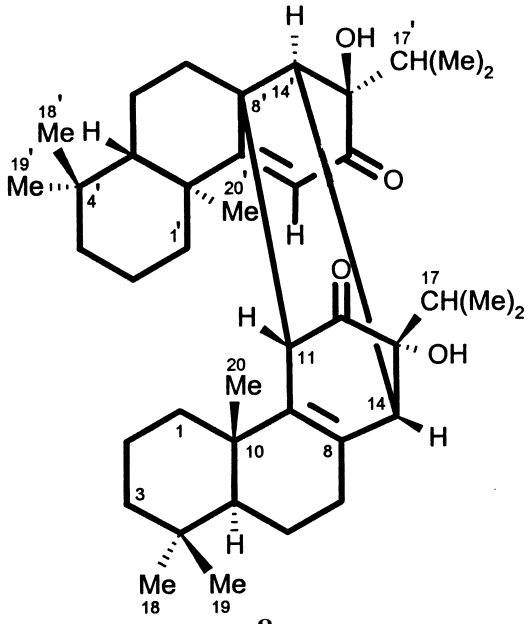
Air dried and pulverised root bark (1.5 kg) was extracted consecutively with *n*-hexane, CH_2Cl_2 and EtOH at room temp. for 2 days, and the extracts were fractioned by VLC. The *n*-hexane extract (4.5 g) was separated by VLC to yield fr No. 1–12. Fr 2 by CC on silica gel and subsequent purification on Sephadex® LH-20 afforded **1** (80 mg). Fr 5 by CC (silica gel), prep. TLC and purification on Sephadex® LH-20 yielded **2** (16 mg) and **3** (8 mg). Separation of the CH_2Cl_2 extract by VLC yielded 12 frs. From fr. 2 compound **1** (20 mg), from fr. 4 compound **2** (44 mg) was isolated by repeated CC on silica gel and Sephadex® LH-20. Frs 6 and 8 were treated in the same way to yield **4** (120 mg) and **5** (24 mg), respectively.

Hugorosenone (3β - *hydroxy* - 1(10),15 - *rosadien* - 2-*one*) (**1**). Crystals. Mp 124–126° (from EtOH). TLC: R_f 0.65; anisaldehyde: blue. $[\alpha]_D$ +27° (*c* 1.6). IR ν_{max} cm^{-1}: 3460, 2970, 2931, 2862, 1670, 757. UV λ_{max} nm: 240 (log ε 4.28). ^1H NMR: Table 1. ^{13}C NMR: Table 2. EIMS *m/z* (rel. int.): 302.2248 $[M]^+$ (1) (calcd for $C_{20}H_{30}O_2$: 302.2246), 273 (27), 320 (20), 135 (21), 105 (25), 41 (100).

18-*Hydroxyhugorosenone* (3β,18-*dihydroxy*-1(10),15-*rosadien*-2-*one*) (**2**). Oil. TLC: R_f 0.46; anisaldehyde: blue. $[\alpha]_D$ +32° (*c* 1.3). IR ν_{max} cm^{-1}: 3430, 2927, 2855, 1669, 757. UV λ_{max} nm: 246 (log ε 4.66). ^1H NMR: Table 1. ^{13}C NMR: Table 2. EIMS *m/z* (rel. int.): 318.2198 $[M]^+$ (1) (calcd for $C_{20}H_{30}O_3$: 318.2195), 303 (5), 300 (6), 275 (21), 273 (25), 231 (23), 153 (45), 151 (81), 149 (22), 135 (27), 105 (29), 41 (100).



6 $R^1 = H$, $R^2 = OH$
7 $R^1 + R^2 = O$



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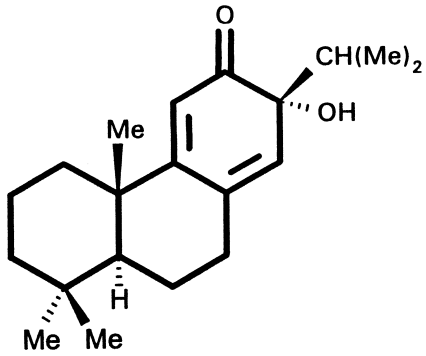

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Hugorosediol (3β,18-*dihydroxy*-1(10),15-*rosadiene*) (**3**). Oil. TLC: R_f 0.43; anisaldehyde: blue. $[\alpha]_D$ +8° (*c* 0.7). IR ν_{max} cm^{-1}: 3338, 2927, 2857, 1379, 1045, 1023, 763. UV: no maximum $\geqslant$ 210 nm. ^1H NMR: Table 1. ^{13}C NMR: Table 2. EIMS *m*/*z* (rel. int.): 304.2406 $[M]^+$ (3) (calcd for $C_{20}H_{32}O_2$: 304.2402), 273 (28), 256 (21), 255 (100), 199 (23), 159 (29), 149 (30), 147 (24), 145 (37), 143 (22), 135 (25), 133 (33), 131 (41), 129 (26), 121 (29), 119 (36), 117 (29), 109 (29), 107 (44), 105 (65).

Hugonone A (**4**). Crystals (120 mg). Mp 151–154°C (from EtOH). TLC: R_f 0.25; anisaldehyde: blue. $[\alpha]_D$ −38° (*c* 0.2). IR ν_{max} cm^{-1}: 3446, 2960, 2874, 1723, 1671, 1156, 1068. UV: λ_{max} nm: 248 (log ε 4.12). ^1H NMR: Table 3. ^{13}C NMR: Table 3. EIMS *m*/*z* (rel. int.): 580.3770 $[M]^+$ (2) (calcd for $C_{36}H_{52}O_6$: 580.3764), 290 (55), 273 (35), 257 (40), 255 (32), 241 (27), 233 (21), 229 (25), 215 (48), 187 (27), 173 (20), 161 (22), 145 (29), 137 (100).

Hugonone B (**5**). Crystals (24 mg). Mp 221–222°C (from EtOH). TLC: R_f 0.38; anisaldehyde: blue. $[\alpha]_D$ −76° (*c* 0.5). IR ν_{max} cm^{-1}: 3444, 2963, 2870, 1722, 1675, 1153, 1065. UV: λ_{max} nm: 248 (log ε 4.08). ^1H NMR: Table 3. ^{13}C NMR: Table 3. EIMS *m*/*z* (rel. int.): 578.3613 $[M]^+$ (3) (calcd for $C_{36}H_{50}O_6$: 578.3607), 290 (89), 288 (28), 273 (35), 271 (21), 257 (73), 255 (21), 248 (32), 246 (29), 245 (33), 233 (36), 231 (84), 230 (24), 229 (48), 215 (78), 213 (30), 211 (22), 199 (24), 189 (38), 187 (40), 185 (32), 173 (34), 161 (37), 159 (32), 147 (39), 145 (39), 138 (28), 137 (100), 135 (23), 133 (24), 131 (30), 129 (23), 128 (23), 121 (26).

Acknowledgements—We thank the Netherlands Organization for International Cooperation in Higher Education (NUFFIC, MHO/Organic Chemistry Project), the Norwegian Agency for International Development (NORAD, Chemistry Project) and the International Foundation for Science (IFS) for financial

Table 3. ^{1}H and ^{13}C NMR data of compounds **4** and **5** (δ in $CDCl_3$, J [Hz])

	4			**5**		
Position	δC	δH	Multiplicity: J	δC	δH	Multiplicity; J
1	28.1	1.55	*	33.7	1.70	*
		1.25	*		1.55	*
2	25.0	1.83	*ddd*; 13,3.5,2.5	34.1	2.54	*ddd*; 16,12,7
		1.60	*		2.40	*ddd*; 16, 6.5,3.5
3	75.6	3.39	*dd*; 3, 2.5	216.4	—	—
4	37.4	—	—	47.3	—	—
5	42.5	1.40	*	49.3	1.50	*
6	18.1	1.55	*	19.5	1.60	*
		1.40	*		1.45	*
7	29.9	2.16	*m*	30.6	2.18	*m*
		1.95	*		2.00	*
8	139.2[a]	—	—	140.5	—	—
9	139.4[a]	—	—	137.5	—	—
10	37.1	—	—	37.0	—	—
11	57.0	3.64	*s*	57.5	3.60	*s*
12	219.9	—	—	212.3	—	—
13	71.8	—	—	71.7	—	—
14	48.9	3.01	*d*; 2.5	49.3	3.08	*d*; 2.5
17	25.9	1.27	*s*	26.0	1.27	*s*
18	27.9	0.97	*s*	26.0	1.10	*s*
19	21.8	0.79	*s*	21.3	0.97	*s*
20	20.4	0.78	*s*	19.6	0.89	*s*
1′	29.9	2.05	*	30.2[a]	1.85	*m*
		1.70	*		1.70	*
2′	25.4	2.05	*	25.5	2.05	*
		1.70	*		1.70	*
3′	75.0	3.50	*br s*	74.8	3.51	*br dd* 2.5, 2.5
4′	38.4	—	—	38.4	—	—
5′	35.1	2.37	*dd*; 12, 7.5	35.2	2.35	*dd*; 12, 7.5
6′	16.0	1.95	*	16.0	1.95	*
		1.55	*		1.55	*
7′	30.5	2.00	*	30.3[a]	2.05	*
		1.95	*		1.95	*
8′	47.8	—	—	47.3	—	—
9′	172.9	—	—	172.7	—	—
10′	41.7	—	—	41.7	—	—
11′	119.9	6.22	*s*	120.0	6.17	*s*
12′	201.0	—	—	201.0	—	—
13′	74.1	—	—	74.1	—	—
14′	47.6	2.42	*d*; 2.5	47.7	2.44	*d*; 2.5
17′	32.4	1.25	*s*	32.3	1.25	*s*
18′	27.6	1.03	*s*	27.6	1.02	*s*
19′	21.6	0.97	*s*	21.6	0.97	*s*
20′	26.4	1.26	*s*	26.4	1.26	*s*

[a] Values might be interchanged; *signals not resolved.

support. We also thank L. B. Mwasumbi and F. M. Mbago of the Herbarium of the University of Dar es Salaam, Department of Botany, for identification of the plant. Thanks are also due to the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie, for financial support.

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