Triterpenoids from the Mangrove Plant *Hibiscus tiliaceus*

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Three new triterpenoids with the rarely occurring nigrum skeleton, namely (20*E*)-22-hydroxynigrum-20-en-3-one (**1**), 21β-hydroxynigrum-22(29)-en-3-one (**2**), and 21α-hydroxynigrum-22(29)-en-3-one (**3**), were isolated from the mangrove plant *Hibiscus tiliaceus*. Additionally, five known triterpenoids including friedelin (**4**), 12-oleanen-3β-ol (**5**), 3β-hydroxy-12-oleanen-28-oic acid (**6**), 20(29)-lupen-3β,28-diol (**7**), and cucurbita-5,23-dien-3β,25-diol (**8**) were also isolated and identified. The latter structures were elucidated by a detailed NMR and MS analyses, as well as by comparison with reported literature data.

Introduction. – *Hibiscus tiliaceus* (Malvaceae) is a pantropical mangrove plant that grows in littoral forests and mangrove forest margins of atolls and high islands. In China, this species is mainly distributed along the southern and southeastern coastlines [1]. This plant is used as traditional medicine in Asia and Africa to treat diseases such as fever, cough, phlegm, and dysentery [2]. Previous investigations centered on *H. tiliaceus* led to the isolation of a series of oleanane- and friedelane-type triterpenoids [2]. As part of our interests in the chemical investigation of mangrove plants [3], a systematic study of the aqueous EtOH extract of *H. tiliaceus* was carried out, and eight triterpenoids, thereof three of the new nigrum-type, namely, (20*E*)-22-hydroxynigrum-20-en-3-one[1]) (**1**), 21β-hydroxynigrum-22(29)-en-3-one[1]) (**2**), and 21α-hydroxynigrum-22(29)-en-3-one[1]) (**3**), and five known triterpenoids, friedelin (**4**) [4], 12-oleanen-3β-ol (**5**) [5], 3β-hydroxy-12-oleanen-28-oic acid (**6**) [6], 20(29)-lupen-3β,28-diol (**7**) [7], and cucurbita-5,23-dien-3β,25-diol (**8**) [8] were isolated. In this paper, we report the isolation and structure determination of the new compounds **1**–**3**.

Results and Discussion. – The dried and powdered mangrove plant *H. tiliaceus* was extracted with 95% aqueous EtOH, and the residue was further extracted with $CHCl_3$/MeOH (1:1) at room temperature. The concentrated extracts were combined, dissolved in H_2O, and extracted with petroleum ether (PE), AcOEt, and BuOH, successively. The AcOEt-soluble fraction was further purified by column chromatography (silica gel, reversed-phase silica gel, and *Sephadex LH-20*) to yield compounds **1**–**8** (*Fig. 1*).

[1]) For systematic names, see *Exper. Part.*

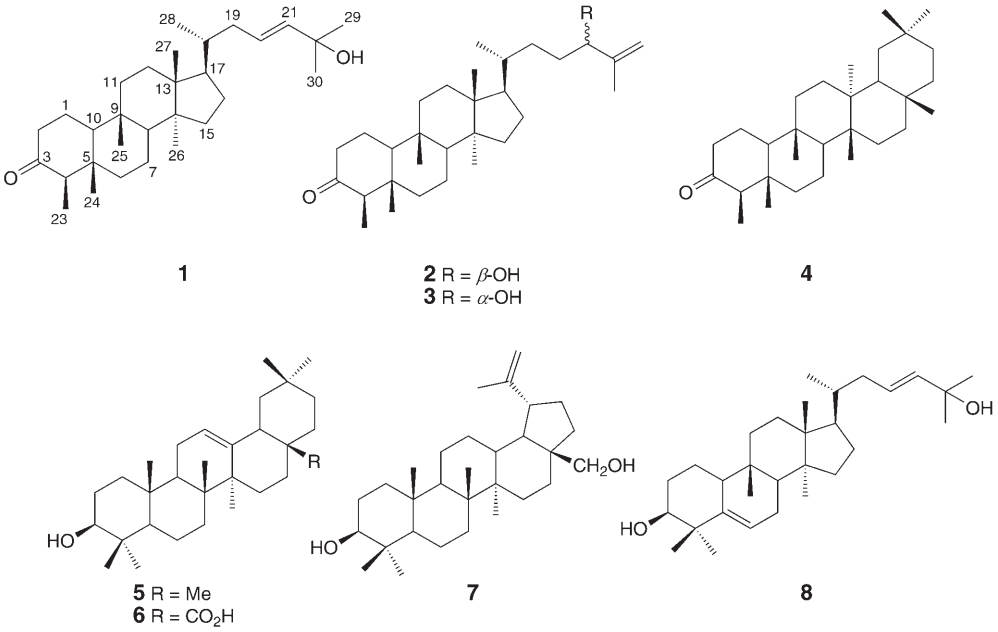

Fig. 1. *Compounds isolated from* H. tiliaceus

Compound **1** was obtained as a colorless oil. The EI-MS showed a characteristic fragment-ion peak at *m/z* 424 ($[M-H_2O]^+$), and the molecular formula was determined to be $C_{30}H_{50}O_2$ by HR-ESI-MS (pos.; 465.3717 ($[M+Na]^+$, $C_{30}H_{50}NaO_2^+$; calc. 465.3708)), suggesting six degrees of unsaturation. The ^{1}H-NMR spectrum showed six Me *singlet*s at δ(H) 0.79 (Me(27)), 0.83 (Me(24)), 1.05 (Me(25)), 1.10 (Me(26)), and 1.31 (Me(29), Me(30)), two Me *doublet*s at δ(H) 0.87 ($J = 6.4$ Hz, Me(28)) and 0.88 ($J = 6.6$ Hz, Me(23)), and two broad *singlet*s assigned to two olefinic H-atoms H–C(20) and H–C(21) at δ(H) 5.59 and 5.60, respectively. The ^{13}C-NMR spectrum revealed the presence of 30 C-atoms which were classified by DEPT experiments as eight Me, nine CH_2, and seven CH groups, and six quaternary C-atoms. Detailed NMR data comparison with the spectra reported for nigrum-21-en-3-one revealed that **1** has a nigrum-type triterpenoid skeleton, and that it differs from nigrum-21-en-3-one only within its side chain [9]. The presence of a $-CH(Me)-CH_2-CH=CH-C(OH)(Me)_2$ unit in the side chain was deduced by the observed correlations in the 2D-NMR experiments and by comparison with reported literature values [8]. In the ^{1}H,^{1}H-COSY spectrum, H–C(18) correlated with the *doublet* of Me(28) and the *multiplet* of CH_2(19). The latter was determined to be connected with the double bond at C(20) by COSY. The position of the OH group at C(22) was deduced by the observed HMBC correlations from Me(29) to C(21), C(22), and C(30), and from Me(30) to C(21), C(22), and C(29). Comparable chemical shifts to those of cucurbita-5,23-dien-3β,25-diol (**8**) [8] further supported the presence of the postulated side chain. The observed HMBC correlations from Me(28) and H–C(18) to C(17) indicated the connection of the side chain to C(17). Further HMBC correlations,

i.e., from Me(23) to C(3), C(4), and C(5), from Me(24) to C(4), C(5), C(6), and C(10), from Me(25) to C(8), C(9), C(10), and C(11), from Me(26) to C(8), C(13), C(14), and C(15), and from Me(27) to C(12), C(13), C(14), and C(17), confirmed the constitutional formula of **1**, as shown in *Fig. 2*.

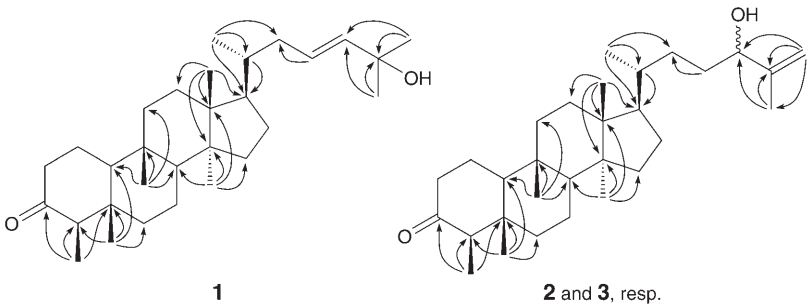

Fig. 2. *Selected HMBC correlations for* **1** *and* **2**/**3**

The relative configuration of **1** was determined by comparison of its NMR data with those of literature reports [8][9]. Me(23), Me(24), Me(25), and Me(27), were in β-orientation, and H–C(17), Me(26) and Me(28) were in α-orientation as deduced by a detailed NMR data comparison with the values reported for nigrum-21-en-3-one [9]. Therefore, the side chain at C(17) was in β-orientation. The double bond was assigned to be *trans*-configured by NMR data comparison with cucurbita-5,23-dien-3β,25-diol (**8**) [8]. Based on the above evidence, the structure of compound **1** was established as (20*E*)-22-hydroxynigrum-20-en-3-one[1]), according to the standard triterpenoid numbering system [9].

Compounds **2** and **3** were obtained as a colorless oily mixture in a 1:1 ratio. They displayed one spot on TLC, and attempts to separate them by column chromatography or by preparative TLC failed with various solvent systems. The EI-MS of **2** (or **3**) showed a characteristic molecular-ion peak at *m*/*z* 442 (M^+) and a fragment-ion peak at *m*/*z* 424 ($[M-H_2O]^+$). The molecular formula was deduced to be $C_{30}H_{50}O_2$ by HR-ESI-MS (pos.; 465.3700 ($[M+Na]^+$, $C_{30}H_{50}NaO_2^+$; calc. 465.3708)), suggesting six degrees of unsaturation. The ^{1}H-NMR spectrum (*Table*) showed five Me *singlet*s at δ(H) 0.79 (Me(27)), 0.83 (Me(24)), 1.05 (Me(25)), 1.09 (Me(26)), and 1.72 (Me(30)), two Me *doublet*s at δ(H) 0.88 (J = 6.6 Hz, Me(23)) and 0.89 (J = 7.0 Hz, Me(28)), and a pair of broad *singlet*s characteristic for terminal olefinic H-atoms at δ(H) 4.83 (H–C(29) and 4.92 (H′–C(29). The ^{13}C-NMR and DEPT spectra (*Table*) of **2** (and **3**) revealed the presence of 30 C-atoms, involving seven Me, eleven CH_2, and six CH groups, and six quaternary C-atoms. Detailed NMR data comparison with the spectra of **1** revealed that **2** (or **3**) possessed a nigrum-type triterpenoid skeleton as well, differing from **1** only in the positions of the double bond and the OH group within the side chain. The OH group in **2** (or **3**) was assigned to be attached to C(21) upon its chemical shift at δ(C) 76.3 (H–C) [10]. This result was supported by the observed HMBC correlations from the oxygenated H–C(21) to C(20), C(22), C(29), and C(30). The terminal double bond was placed at C(22) as established by the HMBC correlations from CH_2(29) to C(21), C(22), and C(30), and from Me(30) to C(21),

C(22), and C(29). The other HMBC correlations, from Me(23) to C(3), C(4), and C(5), from Me(24) to C(4), C(5), C(6), and C(10), from Me(25) to C(8), C(9), C(10), and C(11), from Me(26) to C(8), C(13), C(14), and C(15), from Me(27) to C(12), C(13), C(14), and C(17), and from Me(28) to C(17), C(18), and C(19), further confirmed the structure of **2** (and **3**).

Table. *^{1}H- and ^{13}C-NMR Data of* **1**–**3**. At 500 and 125 MHz, resp., in $CDCl_3$. Assignments were corroborated by ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments.

	1		**2/3**	
	δ(H)	δ(C)	δ(H)	δ(C)
H_a–C(1)	1.68–1.71 (*m*)	24.0 (*t*)	1.69–1.71 (*m*)	24.0 (*t*)
H_b–C(1)	2.12–2.16 (*m*)		2.11–2.16 (*m*)	
H_a–C(2)	2.25–2.39 (*m*)	42.2 (*t*)	2.25–2.39 (*m*)	42.2 (*t*)
H_b–C(2)	2.41–2.44 (*m*)		2.41–2.44 (*m*)	
C(3)		212.9 (*s*)		212.9 (*s*)
H–C(4)	2.25 (*q*, *J* = 6.6)	58.5 (*d*)	2.25 (*q*, *J* = 6.6)	58.5 (*d*)
C(5)		40.5 (*s*)		40.5 (*s*)
H_a–C(6)	1.38–1.40 (*m*)	35.2 (*t*)	1.37–1.38 (*m*)	35.2 (*t*)
H_b–C(6)	1.75–1.78 (*m*)		1.75–1.78 (*m*)	
H_a–C(7)	1.50–1.52 (*m*)	20.2 (*t*)	1.52–1.54 (*m*)	20.2 (*t*)
H_b–C(7)	1.78–1.79 (*m*)		1.77–1.79 (*m*)	
H–C(8)	1.70–1.74 (*m*)	45.3 (*d*)	1.69–1.72 (*m*)	45.3 (*d*)
C(9)		35.9 (*s*)		35.9 (*s*)
H–C(10)	1.97–2.01 (*m*)	49.3 (*d*)	1.97–1.99 (*m*)	49.4 (*d*)
CH_2(11)	1.95–1.98 (*m*)	34.6 (*t*)	1.95–1.98 (*m*)	34.7 (*t*)
H_a–C(12)	1.49–1.50 (*m*)	31.5 (*t*)	1.49–1.52 (*m*)	31.2 (*t*)
H_b–C(12)	1.66–1.69 (*m*)		1.60–1.66 (*m*)	
C(13)		45.8 (*s*)		45.8 (*s*)
C(14)		50.5 (*s*)		50.5 (*s*)
CH_2(15)	1.25–1.27 (*m*)	36.1 (*t*)	1.25–1.27 (*m*)	36.1 (*t*)
CH_2(16)	1.91–1.94 (*m*)	27.7 (*t*)	1.27–1.32 (*m*), 1.89–1.92 (*m*)	27.8/27.7 (*t*)
H–C(17)	1.55–1.64 (*m*)	50.4 (*d*)	1.60–1.63 (*m*)	50.6 (*d*)
H–C(18)	1.49–1.51 (*m*)	36.6 (*d*)	1.25–1.29 (*m*)	36.2/36.1 (*d*)
H_a–C(19)	1.74–1.76 (*m*)	39.1 (*t*)	0.92–0.93 (*m*)	32.0 (*t*)
H_b–C(19)	2.15–2.18 (*m*)			
H–C(20)	5.59 (br. *s*)	125.5 (*d*)	1.38–1.42 (*m*)	31.7/31.8 (*t*)
H–C(21)	5.60 (br. *s*)	139.5 (*d*)	4.02 (*t*, *J* = 6.5)	76.3/76.7 (*d*)
C(22)		70.7 (*s*)		147.8/147.5 (*s*)
Me(23)	0.88 (*d*, *J* = 6.6)	6.8 (*q*)	0.88 (*d*, *J* = 6.6)	6.8 (*q*)
Me(24)	0.83 (*s*)	15.7 (*q*)	0.83 (*s*)	15.7 (*q*)
Me(25)	1.05 (*s*)	28.8 (*q*)	1.05 (*s*)	28.8 (*q*)
Me(26)	1.10 (*s*)	22.6 (*q*)	1.09 (*s*)	22.6 (*q*)
Me(27)	0.79 (*s*)	15.6 (*q*)	0.79 (*s*)	15.6 (*q*)
Me(28)	0.87 (*d*, *J* = 6.4)	18.6 (*q*)	0.89 (*d*, *J* = 7.0)	18.7 (*q*)
Me(29) or CH_2(29)	1.31 (*s*)	30.0 (*q*)	4.83/4.84 (br. *s*), 4.92/4.93 (br. *s*)	111.3/110.9 (*t*)
Me(30)	1.31 (*s*)	29.9 (*q*)	1.72 (*s*)	17.6/17.2 (*q*)

The relative configuration of **2** (and **3**) was determined by comparison with that of **1** and literature reports [9]. The Me(23), Me(24), Me(25), Me(27), and the side chain

at C(17) were in β-orientation and Me(26) and Me(28) were in α-orientation for **2** (or **3**). However, the relative configuration of the OH group at C(21) is presently still unknown. Therefore, the structure for compounds **2** and **3** were established as (21*RS*)-21-hydroxynigrum-22-en-3-one. Similar epimeric mixtures have been reported previously [10].

The nigrum-type triterpenoid skeleton was first reported in 1995 for a compound isolated from *Empetrum nigrum* [9]. To the best of our knowledge, the present investigation represents the first isolation of this type of compounds from mangrove plants. The isolation of compounds **1**–**3** represents a new addition to the molecular diversity of *Hibiscus tiliaceus*, compared with other types of triterpenoids isolated from the same species [2].

Up to now, no biological evaluation for nigrum-type triterpenoids has been reported. In antibacterial and antifungal assays [11], compounds **1**–**3** exhibited no significant activities against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*.

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Experimental Part

General. Column chromatography (CC): commercial silica gel (200–300 and 300–400 mesh; *Qingdao Haiyang Chemical Group Co.*), *Lobar LiChroprep RP-18* (40–63 μm; *Merck*), and *Sephadex LH-20* (*Sigma*). TLC: Precoated silica gel plates *GF-254* (*Qingdao Haiyang Chemical Group Co.*). Optical rotation: *Atago Polax-L* polarimeter. ^{1}H- and ^{13}C-NMR Spectra: *Bruker Avance-500* spectrometer; at 500/125 MHz, resp.; δ in ppm, J in Hz. Low-resolution EI-MS and high-resolution ESI-MS: *VG Autospec-3000* mass spectrometer; in m/z.

Plant Material. The mangrove plant *Hibiscus tiliaceus* LINN. was collected in Hainan Province of Southern China, in December 2005. The plant was identified by Prof. *H. Peng* at the Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (0407G) has been deposited at the Key Laboratory of Experimental Marine Biology of the Institute of Oceanology, Chinese Academy of Sciences.

Extraction and Isolation. The dried and powdered mangrove plant *H. tiliaceus* (2.32 kg) was extracted with 95% aq. EtOH. After solvent removal, the residue was further extracted with $CHCl_3$/MeOH 1:1 at r.t. The concentrated extracts were combined, dissolved in H_2O, and extracted with petroleum ether (PE), AcOEt, and BuOH, successively. The AcOEt-soluble fraction (37 g) was subjected to CC (SiO_2; PE/acetone gradient, then $CHCl_3$/MeOH gradient) to afford 26 fractions (*Fr. 1–26*) according to TLC.

Fr. 1 was dissovled in $CHCl_3$ and left at r.t. to yield colorless crystals of **4** (20.3 mg). *Fr. 8* was rechromatographed by CC (*Sephadex LH-20*, $CHCl_3$/MeOH, 1:1) to yield five subfractions (*Subfrs. 1–5*). *Subfr. 3* (0.5 g) was further purified by CC (*RP-18*; MeOH) to yield **5** (4.2 mg). *Fr. 12* was separated by CC (*Sephadex LH-20*, $CHCl_3$/MeOH 1:1) to afford four subfractions (*Subfrs. 1–4*). *Subfr. 3* was further separated by CC (SiO_2; 300–400 mesh; PE/AcOEt 15:1) to yield a mixture of **2** and **3** (8.8 mg). *Fr. 14* was also submitted to CC (*Sephadex LH-20*, $CHCl_3$/MeOH 2:1) to afford five subfractions (*Subfrs. 1–5*). *Subfr. 2* was further purified by CC (1. SiO_2, 300–400 mesh, PE/AcOEt 15:1; 2. *Sephadex LH-20*, MeOH) to yield **1** (10.8 mg) and **6** (3.7 mg), resp. *Fr. 17* was also subjected to CC (*Sephadex LH-20*, $CHCl_3$/MeOH 2:1) to furnish six subfractions (*Subfrs. 1–6*). *Subfr. 3* was purified by CC (1. SiO_2, 300–

400 mesh, PE/AcOEt 5:1; 2. *Sephadex LH-20*, MeOH; 3. *RP-18*, MeOH) to yield **7** (13.2 mg) and **8** (5.2 mg), resp.

*(20*E*)-22-Hydroxynigrum-20-en-3-one* (*=(4β,5β,9β,10α,17β)-17-[(3*E*)-5-Hydroxy-1,5-dimethylhex-3-en-1-yl]-4,5,9,14-tetramethylestran-3-one*; **1**). Colorless oil. $[\alpha]_D^{22} = -23.8$ ($c = 0.31$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: see *Table*. EI-MS: 424 (15, $[M-H_2O]^+$), 409 (6), 343 (13), 315 (6), 313 (18), 273 (26), 231 (24), 189 (32), 161 (66), 121 (100), 109 (65), 97 (89), 81 (61), 69 (53). HR-ESI-MS (pos.): 465.3717 ($[M+Na]^+$, $C_{30}H_{50}NaO_2^+$; calc. 465.3708).

*(21*RS*)-21-Hydroxynigrum-22(29)-en-3-one* (*=(4β,5β,9β,10α,17β)-17-[(4*RS*)-4-Hydroxy-1,5-dimethylhex-5-en-1-yl)-4,5,9,14-tetramethylestran-3-one*; **2** or **3**). Colorless oil. ^{1}H- and ^{13}C-NMR: see *Table*. EI-MS: 442 (2, M^+), 424 (9, $[M-H_2O]^+$), 409 (4), 357 (2), 315 (9), 288 (14), 273 (24), 231 (22), 189 (30), 121 (41), 109 (48), 97 (100), 81 (52), 69 (30). HR-ESI-MS (pos.): 465.3700 ($[M+Na]^+$, $C_{30}H_{50}NaO_2^+$; calc. 465.3708).

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