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STEROIDS AND TERPENOIDS FROM THE GUM RESIN OF *AILANTHUS GRANDIS*

T. HUNG, H. STUPPNER,* E. P. ELLMERER-MÜLLER,† D. SCHOLZ,‡ D. EIGNER§ and M. P. MANANDHAR¶

Institut für Pharmakognosie der Universität Innsbruck, Josef-Moeller-Haus, Innrain 52, A-6020 Innsbruck, Austria; †Institut für Organische Chemie der Universität Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria; ‡Sandoz Forschungsinstitut, Brunnerstr. 59, A-1235 Wien, Austria; §Institut für Geschichte der Medizin der Universität Wien, Währingerstr. 25 A-1090 Wien, Austria; ¶Department of Forestry and Plant Research, National Herbarium and Plant Laboratory, Godawary, Lalitpur, Nepal

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Key Word Index—*Ailanthus grandis*; Simaroubaceae; pregnane; gammacerane; structure elucidation.

Abstract—From the gum-resin of *Ailanthus grandis* six pregnane, two cholestane, two hopane, one lupane and one gammacerane derivatives have been isolated. One, 3-α-acetyloxy-5-α-pregnan-16-one, is a new compound. Four, 20*S*-acetyloxy-4-pregnene-3,16-dione, 16-β-acetyloxy-pregn-4,17(20)-*trans*-dien-3-one, gammacerane-3,21-dione and 3-α-acetyloxy-5-α-pregn-17(20)-(*cis*)-en-16-one, are new natural products. Their structures were established by UV, IR, MS, ^{1}H and ^{13}C NMR spectroscopy and two-dimensional NMR techniques (COSY, HMQC and HMBC).

INTRODUCTION

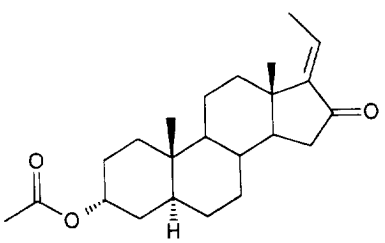
Ailanthus grandis Prain is a lofty tree (30–45 m in height) growing in India, Vietnam, Thailand and China [1–3]. In traditional medicine in Nepal and Northern India, the gum-resin from the trunk of this plant (known as 'Gokuldhup') has been used for the treatment of boils and pimples (M. P. Manandhar, personal communication).

Previous phytochemical investigations of *A. grandis* resulted in the isolation of two quassinoids, 6-α-tigloyloxy-chaparrinone and 6-α-tigloyloxy-chaparrin, which exhibited significant cell growth inhibition in the P-388 cell line assay [4, 5]. No phytochemical, or pharmacological data have been available so far for the gum resin.

This communication refers to the isolation and structure elucidation of five new natural compounds: 16-β-acetyloxy-pregn-4-17(20)-*trans*-dien-3-one (**1**), 3-α-acetyloxy-5-α-pregn-17(20)-(*cis*)-en-16-one (**2**), 3-α-acetyloxy-5-α-pregnan-16-one (**3**), 20*S*-acetyloxy-4-pregnene-3,16-dione (**4**), gammacerane-3,21-dione (**5**). Also studied are *Z*- (**6**) and *E*-guggulsterone, guggulsterol I [6], 22-hydroxy-hopanone-3 [7–9], hop-17(21)-ene-3-one [10], cholest-4-ene-3-one [11, 12], lup-20(29)-ene-3-one-16-ol (resinone) [13], 1,5,9-trimethyl-1,5,9-cyclododecatriene and cembrene [14, 15].

RESULTS AND DISCUSSION

Fractionation of the gum resin exudate from *A. grandis* by gel filtration (Sephadex LH 20), silica gel and reversed phase chromatography, afforded the compounds **1**–**14**.



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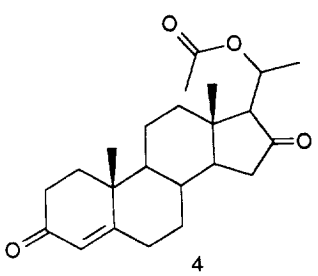

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Guggulsterone (pregn-4,17(20)-*trans*-diene-3,16-dione) (**6**), one of the major constituents of the gum-resin, was identified by X-ray analysis. Although this compound has been described in the literature as a constituent of the gum resin of *Commiphora mukul* (Hook. ex Stocks) Engl. (Burseraceae) [6], neither X-ray data nor complete NMR spectral data have been available. A perspective drawing of the compound is given in Fig. 1, the complete NMR assignments are shown in Tables 1 and 2.

The CI and EI mass spectra of **1** displayed $[M + H]^+$ and $[M]^+$ peaks at *m/z* 357 and 356, respectively, indicating the molecular formula of $C_{23}H_{32}O_3$. The

*Author to whom correspondence should be addressed.

^{13}C NMR spectrum showed the presence of 23 carbon signals. Six of these carbons were quaternary, six were tertiary, seven were secondary and four were primary. The spectra also exhibited the presence of a carbonyl carbon (δ199.3), two double bonds (–CH=C <) (δ124.0, 170.7 and 118.4, 148.7), a carbinyl carbon (δ72.9) and one carboxyl carbon (δ170.7) assignable to an acetyl group (δ_C21.1; δ_H2.02) [16]. The presence of an acetyloxy group was also supported by the lack of a free OH peak in the range 3600–3000 cm^{-1} in the IR spectrum. The complete structure of **1** was established by means of 2D NMR spectroscopy. The double bond was established in positions C-4 and C-5 by an HMBC experiment, which showed cross-peaks between the sp^2 quaternary carbon C-5 and the C-19 methyl protons, as well as the vinylic proton in C-4 and the quaternary carbon C-10. The ketone carbon belonging to the enone system was located at C-3. The C-4 proton appeared as a small doublet at δ5.73 (J = 1.7 Hz) coupling with the *axial* proton of an allylic methylene group at C-6. The C-2 protons, which were in the vicinity of the ketone function, in the ^{1}H–^{1}H COSY spectrum clearly showed cross-peaks with the protons of C-1. The second double bond

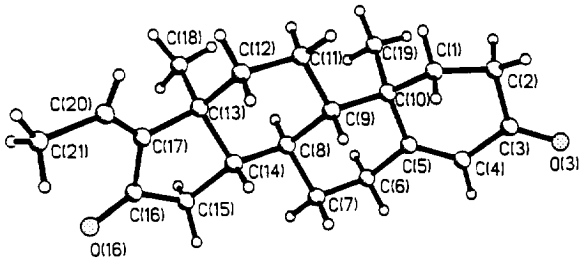

Fig. 1. Perspective view of the structure of compound **6**.

Table 1. ^{1}H NMR spectral data of compounds **1**–**6** ($CHCl_3$-*d*, δ ppm)*

H	1	2	3	4	5	6
1a	1.7 *m*	1.23 *m*	1.23 *m*	1.72 *m*	1.4 *m*	1.9 *m*
1b	2.00 *m*	1.5 *m*	1.50 *m*	2.00 *m*	1.91 *ddd*	2.05 *m*
2a	2.32 *m*	1.60 *m*	1.64 *m*	2.4 *m*	2.43 *m*	2.4 *m*
2b	s.o.	1.72 *m*	1.71 *m*	2.4 *m*	2.43 *m*	2.5 *m*
3		5.01 *quin*	5.00 *quin*			
4a	5.73 *d* (1.7)	1.5 *m*	1.48 *m*	5.07 *s*		5.70 *m*
4b		1.5 *m*	1.56 *m*			
5		1.5	1.47		1.3 *m*	
6a	2.24 *m*	1.22 *m*	1.20 *m*	2.34 *dd*	1.5 *m*	2.3 *m*
6b	2.24 *m*	s.o.	s.o.	2.4 *m*	1.5 *m*	2.42 *m*
7a	1.01 *m*	1.63 *m*	1.63 *m*	1.1 *m*	1.32 *m*	1.1 *m*
7b	1.83 *m*	s.o.	1.60 *m*	1.8 *m*	1.5 *m*	1.80 *m*
8	1.60 *m*	1.5 *m*	1.52 *m*	1.71 *m*		1.70 *m*
9	0.92 *m*	0.90 *m*	0.92 *m*	1.1 *m*	1.34 *m*	1.1 *m*
11a	1.5 *m*	1.31 *m*	1.32 *m*	1.67 *m*	1.34 *m*	1.50 *m*
11b	1.62 *m*	1.7 *m*	1.63 *m*		1.6 *m*	1.72 *m*
12a	1.20 *m*	1.34 *m*	1.34 *m*	1.4 *m*	1.34 *m*	1.4 *m*
12b	1.83 *m*	1.9 *m*	1.89 *m*	2.00 *m*	1.6 *m*	s.o.
13					1.34 *m*	
14	0.90 *m*	1.4 *m*	1.44 *m*	1.41 *m*		1.4 *m*
15a	1.26 *m*	2.21 *m*	1.73 *m*	2.00 *m*	1.32 *m*	2.20 *m*
15b	2.40 *m*	s.o.	2.20 *m*	2.20 *m*	1.5 *m*	s.o.
16a	5.60 *t* (7.3)				1.5 *m*	
16b					1.5 *m*	
17			1.64 *m*	2.0 *m*	1.3 *m*	
18	0.94 *s*	0.83 *s*	0.67 *s*	0.81 *m*		0.93 *s*
19a	1.2 *s*	1.00 *s*	0.81 *s*	1.2 *s*	1.4 *m*	1.21 *s*
19b					1.91 *ddd*	
20a	5.32 *dq* (1.6 7.6)	6.58 *q* (7.3)	1.22 *m*	5.10 *q* (6.1)	2.43 *m*	5.7 *q* (7.1)
20b			1.63 *m*		2.43 *m*	
21	1.6 *d* (7.6)	1.83 *d*	1.00 *t* (7 3)	1.33 *d*		2.1 *d* (7.1)
23/30					1.1 *s*	
24/29					1.02 *s*	
25/28					0.92 *s*	
26/27					1.0 *s*	
CH_3CO	2.03 *s*	2.03 *s*	2.03 *s*	2.0 *s*		

*Assignments were confirmed by 2D ^{1}H COSY, HMQC and HMBC experiments.

†Coupling constants (J, Hz) are given in parentheses; s.o.: signal totally obscured by other signals.

Table 2. ^{13}C NMR data of compounds **1**–**6** (in $CHCl_3$-*d*, δ ppm)*

C	1	2	3	4	5	6
1	35.7 *t*	32.9[a] *t*	32.8[a] *t*	35.5 *t*	39.5 *t*	35.5 *t*
2	33.9 *t*	26.1 *t*	26.1 *t*	33.8 *t*	34.1 *t*	33.8 *t*
3	199.3 *s*	70.0 *d*	70.0 *d*	199.0 *s*	217.8 *s*	199.1 *s*
4	124.0 *d*	32.6[a] *t*	32.7[a] *t*	124.2 *d*	47.3 *s*	124.1 *d*
5	170.7 *s*	40.0 *d*	40.1 *d*	170.4 *s*	54.9 *d*	170.2 *s*
6	32.7 *t*	28.1 *t*	28.1 *t*	32.5 *t*	19.6 *t*	32.5 *t*
7	31.4 *t*	31.9 *t*	32.1 *t*	31.9 *t*	32.5 *t*	31.8 *t*
8	35.0 *d*	34.2 *d*	34.5 *d*	34.2 *d*	41.8 *s*	34.6 *d*
9	54.0 *d*	54.0 *d*	54.3 *d*	53.4 *d*	49.7 *d*	53.6 *d*
10	38.7 *s*	36.0 *s*	36.0 *s*	38.6 *s*	36.8 *s*	38.7 *s*
11	20.7 *t*	20.6 *t*	20.3 *t*	20.2 *t*	21.8 *t*	20.6 *t*
12	35.8 *t*	36.4[b] *t*	38.3[b] *t*	38.0 *t*	21.8 *t*	35.4 *t*
13	43.1 *s*	43.4 *s*	42.1 *s*	41.7 *s*	49.7 *d*	43.0 *s*
14	50.8 *d*	50.2 *d*	50.7 *d*	49.9 *d*	41.8 *s*	4.0 *d*
15	33.2 *t*	37.9[b] *t*	38.5[b] *t*	38.8 *t*	32.5 *t*	39.2 *t*
16	72.9 *d*	206.4 *s*	219.5 *s*	213.9 *s*	19.8 *t*	207.2 *s*
17	148.7 *s*	148.1 *s*	63.4 *d*	65.9 *d*	54.9 *d*	147.8 *s*
18	19.0 *q*	17.7 *q*	13.4[c] *q*	13.6 *q*	36.8 *s*	19.5 *q*
19	17.4 *q*	11.1 *q*	11.4 *q t*	17.3 *q*	39.5 *t*	17.3 *q*
20	118.4 *d*	128.9 *d*	17.6 *t*	67.0 *d*	34.1 *t*	130.4 *d*
21	13.4 *q*	13.1 *q*	13.5[c] *q*	19.9 *q*	217.8 *s*	14.0 *q*
22					47.3 *s*	
23					21.1 *q*	
24					26.6 *q*	
25					15.9 *q*	
26					16.2 *q*	
27					16.2 *q*	
28					15.9 *q*	
29					26.6 *q*	
30					21.1 *q*	
MeCO	170.7 *s*	170.6 *s*	170.6 *s*	169.8 *s*		
MeCO	21.1	21.5 *q*	21.5 *q*	21.3 *q*		

*Assignments were confirmed by DEPT and 2D ^{1}H-^{13}C one-bond as well as long-range correlation experiments; assigments may be interchangeable within vertical column.

was located between C-17 and C-20. The acetyloxy group was positioned at C-16 by its ^{1}H–^{1}H and ^{1}H–^{13}C interactions. The configuration was established as *trans* (*Z*) by comparing the chemical shift values for the C-18 methyl group of **1** with the corresponding NMR data of *Z*- (**6**) and *E*-guggulsterone. According to Benn and Dodson [16], the orientation of the acetyloxy group at C-16 in the *Z*-isomer can be established by the ^{1}H NMR shift value of the C-18 methyl group. In comparison with the 16-oxo-derivative, an α-oriented acetyloxy group at C-16 causes a larger downfield shift (0.18 ppm) than a β-oriented one (nearly unaffected < 0.06 ppm). The shift difference between H-18 of **1** and H-18 of *Z*-guggulsterone (**6**) was 0.009 ppm. Consequently, the orientation of the acetyloxy group at C-16 was determined as β. The structure of **1** was thus identified as 16-β-acetyloxy-pregn-4,17(20)-*trans*-dien-3-one. The unambiguous assignments for carbons and protons of **1** are shown in Tables 1 and 2, ^{1}H–^{13}C long-range connectivities in Fig. 2. Compound **1** has been known only as a partially synthetic compound [16]; NMR data have not been reported.

The CI mass spectrum of the acetyloxy-pregnen-one derivative (**2**) showed a $[M + H]^+$ peak at *m/z* 359, indicating the molecular formula $C_{23}H_{34}O_3$. Other prominent fragments appeared at *m/z* 299 $[M + H - HOAc]^+$, 203, 177, 149, 107 and 73. ^{1}H and ^{13}C NMR data (Tables 1 and 2) exhibited the presence of a $>C=CH-$ group (δ_C148.1, 128.9; δ_H6.5, 1H, *q*, $J = 7.3$ Hz), a ketone function in a 5-membered ring (δ_C206.4) an acetyl group (δ_C170.6 and 21.5; δ_H2.03 (3H, *s*)) and one carbinyl carbon (δ_C70.0). Placement of the ketone function in position C-16 and the double bond between C-17 and C-20 was established by comparison of the NMR data with those of *E*- and *Z*-guggulsterone. The appearance of a vinyl proton (H_{20}) as a quartet at δ6.48 ($J = 7.3$ Hz) and a doublet of three methyl protons (C-21 methyl group) at δ1.83 ($J = 7.3$ Hz) in the ^{1}H NMR spectrum confirmed this assignment. The *cis* (*E*)-configuration of the C-17 (20) double bond was established by comparison of the ^{13}C NMR shift value of the C-18 methyl carbon signal with that of *E*-guggulsterone. ^{1}H and ^{13}C NMR data for ring C, D and side chain carbons were in good agreement with those of

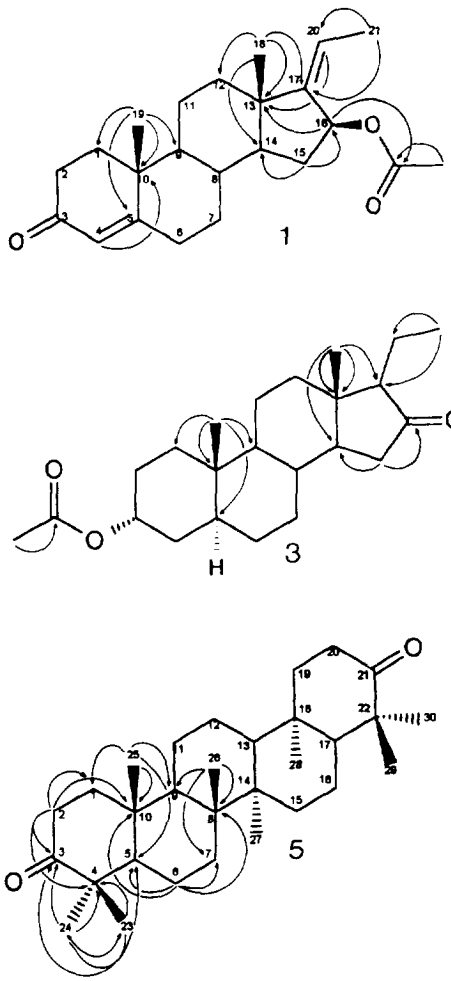

Fig. 2. Most significant correlations observed in HMBC spectra of compounds 1, 3 and 5.

E-guggulsterone. The stereochemistry of the ring A/B junction was deduced from the carbon chemical shift of the C-19 methyl group. It is well known that the C-19 methyl signal of A/B *trans*-steroids is upfield shifted by 11–12 ppm compared with that of their 5β-counterparts, the C-19 resonance of which appears around δ22–24 [17, 18]. The C-19 methyl carbon signal appeared at δ11.1, indicating that **2** belonged to the 5α-pregnane series. The acetyloxy group was located at C-3 of the ring A by comparison with 5α-pregnane, taking into account the α and β effects of the OAc group to vicinal carbons. Accordingly, the carbon signals of C-1, C-2, C-4, and C-5 of **2** are shifted by − 6.1, 3.7, 3.4 and − 7.2 ppm, respectively, relative to those of 5α-pregnane [11]. The configuration of the acetyloxy group was established by the ^{1}H NMR spectrum with the half-height band width ($W_{1/2}$) of 7.1 Hz. This coupling pattern requires the interactions of an equatorial proton (β-oriented in this case) with four vicinal protons [19, 20]. The acetyloxy group was therefore established as an α-oriented one. Assignments for the carbons in rings A and B were in excellent agreement with those for equivalent structures in 5α-steroid series [11]. Therefore, compound **2** is 3α-acetyloxy-5α-preg-17(20)-*cis*-en-16-one. There is only one report dealing with partial synthesis of this compound [21]. This is the first time this compound has been found as a constituent of a plant.

The CI mass spectrum of **3** showed a $[M + H]^+$ peak at *m/z* 361, indicating the molecular formula of $C_{23}H_{36}O_3$. Other prominent fragments appeared at *m/z* 343 and 301 $[M + H - HOAc]^+$. The ^{13}C NMR spectrum of **3** in comparison with that of **2** showed the lack of a double bond in the side chain. This was supported by the appearance of three methyl protons as a triplet (δ_H = 1.00, J = 7.3 Hz) and the absence of the vinyl proton signal in the ^{1}H NMR spectrum. Of the functional carbons, only one ketone function (δ_C219.5), one carbinyl carbon (δ_C70.0) and one acetyl group (δ_C170.6, 21.5; δ_H2.03 (3H, *s*) were observed. The location of the acetyloxy group was established at C-3 by comparing the carbon resonances of the rings A and B with those of **2** (Table 2). The A/B ring junction was determined as *trans*- (i.e. 5α-pregnane derivative) by the shift value of the C-19 methyl carbon. The acetyloxy group in position C-3 was established as an α-oriented substitute from the fact that the pseudoquintet of its geminal proton again showed a relative small half-height band width ($W_{1/2}$ = 7.5 Hz). Assignments for carbon shift values of **3** were achieved by the aid of 2D NMR spectroscopy. The ketone function was localized at C-16. In the HMBC spectrum, two protons of the C-15 methylene carbon (δ_H1.73, 2.20) showed cross-peaks with signals of the carbonyl carbon (δ_C219.5) and the signals of the methine carbon assigned to C-14. The carbon and proton shift values are shown in Tables 1 and 2. Thus, compound **3** is 3α-acetyloxy-5α-pregnan-16-one. The 3β-acetyloxy isomer has been obtained by chemical synthesis [21]; the 3α-acetyloxy isomer to our knowledge is a new compound.

The molecular formula of **4** was determined as $C_{23}H_{32}O_4$ based on CIMS (*m/z* 373 $[M + H]^+$). Inspection of the ^{1}H and ^{13}C NMR data showed that **4** has the same pregnene skeleton as *Z*-guggulsterone (**6**) with carbonyl functions in positions C-3 and C-16, methyl groups at C-10, C-13 and C-20 and a 4,5 double bond. In comparison to guggulsterone, compound **4** has an additional acetyloxy group at C-20 and no 17, 20 double bond. The position of the acetyloxy group was located by an ^{1}H–^{1}H COSY experiment. The C-21 methyl protons showed a coupling, with one proton of the methine carbon bearing an oxygen atom (δ_C67.0). This proton (δ_H5.10, J = 6.4 Hz) in turn coupled with the methine proton assigned to C-17 (δ_H2.00; δ_C65.9). The complete assignments of carbon and proton shift values of **4** are shown in Tables 1 and 2. The 20*S*-stereochemistry of **4** was established by comparing the ^{13}C NMR shift values of C-20 (δ67.0), C-21 (δ19.9) and C-17 (δ65.9) with the corresponding signals of 3β,20*S*-diacetyloxy-5-α-pregnan-16-one [22]. Thus, the structure of **4** is 20*S*-acetyloxy-4-pregnene-3,16-dione. Although **4** has been partially synthesized from 20-hydroxy-4-pregnene-3,16-dione [23], this is the first report of the isolation of this

compound from natural sources. It is also the first report of its ^{13}C NMR data.

The FAB mass spectrum of compound **5** (*m/z* 447 $[M + Li]^+$) suggested the molecular formula of $C_{30}H_{48}O_2$ and the presence of a triterpenic skeleton. Although the molecular formula was attributable to a triterpene, the ^{13}C and ^1H NMR spectra of **5** revealed only 15 carbon and 24 hydrogen signals involving four angular methyl groups, five methylene, two tertiary and four quaternary carbons. The existence of a signal at δ217.8 in the ^{13}C NMR spectrum indicated that one quaternary carbon belonged to a ketone function group. The lack of signals in the range of δ100–150 in the ^{13}C NMR spectrum and in the range higher than δ2.6 in the ^1H NMR spectrum indicated the absence of carbon–carbon double bonds. The results from the NMR spectra corresponded to a formula of $C_{15}H_{24}O$, a half molecule of **5**. This indicated that **5** has a symmetric structure [24, 25]. The complete structure of the 'half molecule' of **5** was obtained from 2D NMR experiments. The HMBC spectrum showed cross-peaks between ^1H and ^{13}C NMR signals of two methyl groups (δ_H1.02, δ_C26.6 and δ_H1.06, δ_C21.1), which could be assigned to C-24 and C-23, respectively [26, 27]. In addition, the two methyl group signals showed cross-peaks with the carbon resonances of a quaternary carbon (δ_C47.3), a keto-carbon (δ_C217.8) and a methine carbon (δ_C54.9). Thus, the methyl groups had to be located at the C-4 position and the ketone group and the methine carbon could be established as C-3 and C-5, respectively, which are usual for triterpenic compounds. The proton signal of another methyl group (δ_H0.92, δ_C15.9), which showed a cross-peak with the C-5 methine carbon resonance, was assignable to C-25. This was confirmed by additional cross-peaks with another tertiary (δ_C49.7), a quaternary (δ_C36.8) and a methylene carbon (δ_C39.5) attributable to C-9, C-10 and C-1, respectively. Similarly, the position of the C-26 methyl group was established. Protons of this group exhibited interactions with the carbons C-9, C-7 and C-8. Signal assignments to C-1, C-2 and C-6 were also achieved by 2D NMR spectra. In the ^1H–^1H COSY spectrum the two protons of C-2 in the vicinity of the ketone group (δ_H2.43, 2H, *m*) showed couplings with the two protons of C-1 (δ_H1.91 and 1.39). In addition, in the HMBC spectrum, the ^1H NMR signals of C-2 revealed a cross-peak with the carbon resonances of C-3, C-1, C-4 and C-10. A carbon that resonated at δ19.8 (δ_H1.46) was assigned to C-6 because its proton signal showed cross-peaks with the ^{13}C NMR resonances of C-5, C-7, C-8 and C-10. The remaining methylene carbon signal (δ_C21.8, δ_H1.34, 1.55, *m*) could be assigned to C-11. Thus, compound **5** is gammaceran-3,21-dione. Assignments of all ^1H and ^{13}C NMR data are shown in Tables 1 and 2. A literature survey indicated that only two reports [28, 29] have dealt with the partial synthesis of gammaceran-3,21-dione by oxidation of a corresponding diol. To the best of our knowledge, this is the first report of the isolation of **5** from natural sources. This is also the first time the ^1H and ^{13}C NMR shift values of **5** are documented.

The occurrence of *Z*-(**6**) and *E*-guggulsterone (**7**) together with guggulsterol-I in the gum resins of *A. grandis* and *C. mukul* may show a certain chemical relationship between the Simaroubaceae and the Burseraceae families. From the botanical point of view, they are closely related families. However, both resins can easily be distinguished by their terpenic constituents, such as hopane, lupane and gammacerane derivatives, which are present in the resin of *A. grandis* but absent in that of *C. mukkul*. The presence of cholestane derivatives, especially 20,22-dihydroxy-cholestan-4-ene-3-one, in the resin of *A. grandis* supports the biosynthetic pathway of C_{21}-steroids in plants from cholestane derivatives via a 20,22-dihydroxy intermediate [6, 30–33]. In nature, gammacerane derivatives (e.g. compound **5**) have been found in crude petroleum, petroleum source rocks and geological sediments. In the plant kingdom they are found only rarely [24].

EXPERIMENTAL

^1H and ^{13}C NMR spectra (δ, ppm, *J* in Hz) were recorded with a Bruker AM 300 (300/75 MHz) using $CHCl_3$-*d* as solvent and internal standard. EIMS spectra were recorded on a Mat 44/S (Finigan) or a Kratos MS 80 RFA mass spectrometer. Chemical ionization (CI) mass spectra were run on the Mat 4415. Fast atom bombardment mass spectra (FABMS) were performed on a Kratos MS 80 RFA mass spectrometer. Single crystal X-ray diffraction experiment was performed on a Siemens R3 m/V diffractometer using CuKα radiation (l = 1.54178 Å) with a balance filter monochromator. Data collection was done at 296 K from an orthorhombic crystal using ω scan technique with 2ϕ range of 0.0°–114.0°. Structure was solved by direct method using a Siemens Shelxtl plus (PC version) program system. Melting points are uncorrected. UV spectra were recorded either in MeOH on a Shimadzu UV-160A or on-line by photo diode array detection (Diode array detector L-4500, Merck-Hitachi) in MeCN/H_2O mixtures. Fourier transform infrared spectroscopy (FT-IR) was performed on a Bruker IFS 25 spectrometer connected to a Bruker infrared microscope.

Column chromatography used silica gel (230–400 mesh) (Merck), Sephadex LH 20 (Pharmacia) (MeOH). TLC: silica gel 60 F_{254}, 0.25 mm (Merck), solvent mixtures: *n*-hexane, *n*-hexane–EtOAc (2:1, 4:1), $CHCl_3$–MeOH (9:1). Spray reagents: vanillin (1%) and H_2SO_4 (10%) in EtOH, followed by heating, Dragendorff's reagent. PLC (preparative-layer chromatography): silica gel 60 F_{254}, 1 mm (Merck), solvent mixture: CH_2Cl_2–EtOAc (19:1). Analytical HPLC was carried out with a Supersphere® 100 RP-18 Lichrocart® 250-4, particle size 5 μm, column, Merck; gradient system: 5 min at 48% MeCN, 48–53% MeCN in 15 min, 53–58% MeCN in 10 min, 58–100% MeCN in 30 min; UV detection was performed at 210 nm. MPLC used Lichroprep® RP-18 silica gel (particle size 40–63 μm, Merck N°13900) as stationary and a mixture of H_2O–MeCN (4:21) as mobile phase.

Plant material. The gum-resin exudate of *A. grandis* was collected in northern India. It was supplied and botanically identified by Dr Manandhar (Department of Forestry and Plant Research, National Herbarium and Plant Laboratories, Godawary, Lalitpur, Nepal). A herbarium specimen is deposited at the herbarium of the Institut für Pharmakognosie, Universität Innsbruck (A).

Isolation of compounds. Ground resin (100 g) was exhaustively extracted with MeOH (5 × 250 ml) in an ultrasonic bath at room temp. for 15 min. The clear solns after filtering were combined. MeOH was removed *in vacuo* to give 41.5 g of a brown, half-solid residue (41 g), which was redissolved in 250 ml of a mixture of MeOH–H_2O (95:5) and then distributed (12 ×) with 100 ml *n*-hexane presaturated with MeOH–H_2O. The hexane partitions were pooled together and the combined soln was washed with 25 × 2 ml MeOH–H_2O and dried over anhydrous Na_2SO_4. The solvent was evaporated under red. pres. to give 23.8 g of residue as a yellow thick oil. A sample (23.5 g) was dissolved in a sufficient amount of MeOH to obtain a thick soln. This soln was subjected to Sephadex LH-20 CC (1 ml for each separation). Frs of 10 ml were collected and monitored by TLC. Frs having the same TLC pattern were pooled. MeOH was removed *in vacuo* to give seven frs, A1 to A7. Among these, frs A4 (13.5 g) and A3 (7.5 g) were the two largest. Fr A4 (13 g) was chromatographed on silica gel. Mixtures of hexane and EtOAc (95:5, 9:1, 4:1, 7:3, 3:2 and 1:1), $CHCl_3$ and then $CHCl_3$–MeOH (8:2) were used for elution. Frs having the same TLC patterns were combined and the solvent removed under red. pres. In all, 22 subfrs were obtained as gummy or thick oil residues, four of them yielding after crystallization (EtOAc) pure compounds **6** and **5**. Silica gel CC of subfr. 19 (300 mg) using CH_2Cl_2 and increasing amounts of EtOAc (3, 5 and 10%) and CH_2Cl_2–EtOH; (9:1) as solvent mixtures gave compound **4** which was purified by repeated silica gel CC using $CHCl_3$ and $CHCl_3$–MeOH; (99:1, 49:1), $CHCl_3$–EtOAc; (99:1, 95:5) and *n*-hexane–EtOAc; (19:1 to 4:1) (yield of **6**). Pure compounds **3** (25 mg) and **2** (3.5 mg) were obtained from subfr. 7 (490 mg) by repeated silica gel CC using CH_2Cl_2 with increasing amounts of EtOAc (from 0 to 2%). Subfr. 13 (1.3 g) was subjected to repeated silica gel CC. Elution was carried out with $CHCl_3$–EtOAc; (24:1) and CH_2Cl_2–C_6H_6; (9:1) with increasing amounts of EtOAc (1% up to 15%). Compound **1**, obtained as a mixture with two other compounds (220 mg) was repeatedly chromatographed on reversed-phase MPLC using an azeotropic mixture of H_2O–MeCN as eluent to give the pure substance (15.8 mg).

Compound **1**. Needles; mp 158–160°; λ_{max} nm: 242 (MeCN–H_2O); IR ν_{max} cm^{-1}: 2963, 2937, 2914, 2855, 2836, 1731, 1672, 1613, 1449, 1438, 1373, 1250, 1235, 1190, 1036, 954, 878. CIMS *m/z* (rel. int.): 357 $[M + 1]^+$ (100), 297 $[M + H - HOAc]^+$ (59.5), 147 (5.8), 113 (4.9), 107 (7.9), 73 (25.6); EIMS *m/z* (rel. int.): 356 $[M]^+$ (1.2), 314 (18.9), 299 (30.9), 281 (14.8), 230 (5.9), 173 (12.2), 91 (27.1), 43 (100); ^1H and ^{13}C NMR data see Tables 1 and 2.

Compound **2**. Slightly yellow crystals; mp 102–103°; UV λ_{max} nm: 243 (MeCN–H_2O); IR ν_{max} cm^{-1}: 2929, 2882, 2856, 1730, 1649, 1590, 1445, 1371, 1259, 1239, 1193, 1150, 1028, 1013, 980, 924, 852. CIMS *m/z* (rel. int.): 359 $[M + H]^+$ (100), 299 $[M + H - HOAc]^+$ (84.1), 203 (1.3), 177 (1.2), 149 (2.0), 107 (1.4), 73 (9.5): ^1H and ^{13}C NMR data see Tables 1 and 2.

Compound **3**. Needles; mp 102–103°; UV λ_{max} nm: 243 (MeCN–H_2O); IR ν_{max} cm^{-1}: 2973, 2938, 2879, 2847, 2865, 1738, 1727, 1650, 1447, 1385, 1364, 1266, 1250, 1166, 1201, 978. CIMS *m/z* (rel. int.): 361 $[M + H]^+$ (46.6), 343 (4.1), 301 $[M + H - HOAc]^+$ (100), 283 (6.1), 203 (2.1), 137 (1.1), 73 (11.4); ^1H and ^{13}C NMR data see Tables 1 and 2.

Compound **4**. Cubic crystals; mp 191–193°; UV λ_{max} nm: 241 (MeOH); IR ν_{max} cm^{-1}: 2979, 2943, 2924, 2857, 1733, 1673, 1610, 1457, 1436, 1419, 1367, 1257, 1183, 1101, 1047, 957; CIMS *m/z* (rel. int.): 373 $[M + H]^+$ (6.4), 313 $[M + H - HOAc]^+$ (100), 312 (23.8), 61 (1.0); ^1H and ^{13}C NMR data see Tables 1 and 2.

Compound **5**. Crystals; mp 317–318°; IR ν_{max} cm^{-1}: 3011, 2992, 2968, 2947, 2863, 1707, 1482, 1456, 1423, 1387, 1377, 1309, 1225, 1142, 1114, 1070, 1005; FABMS *m/z*: 447 $[M + Li]^+$; ^1H and ^{13}C NMR data see Tables 1 and 2.

Compound **6**. Large prisms; mp 189–190°; UV λ_{max} nm: 241 nm (MeOH); IR ν_{max} cm^{-1}: 2963, 2937, 2914, 2855, 1732, 1627, 1313, 1449, 1438, 1373, 1250, 1235, 1036; CIMS *m/z* (rel. int.): 313 $[M + H]^+$ (100), 297 (1.9), 270 (1.2); ^1H and ^{13}C NMR data see Tables 1 and 2.

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REFERENCES

1. Nooteboom, H. P. (1962) *Flora Malesiana Ser. I* **6**, 215.
2. Hegi, G. (1975) in *Illustrierte Flora von Mitteleuropa*, Vol. 5, pp. 80–87. Verlag Paul und Parey, Berlin.
3. Basak, R. K. (1980) in *Fascicles of Flora of India* Fascicle 4, pp 1–7. Botanical Survey of India, Botanic Garden.
4. Seida, A. A., Kinghorn, A. D., Cordell, G. A. and Farnsworth, N. R. (1978) *J. Nat. Prod.* **41**, 584.
5. Polonsky, J., Varon, Z. and Moretti, C. (1980) *J. Nat. Prod.* **43**, 503.
6. Patil, V. D., Nayak, U. R. and Dev, S. (1972) *Tetrahedron* **28**, 2341.
7. Dunstan, W. J., Fazakerley, H., Halsall, T. G. and Jones, E. R. H. (1957) *Croat. Chem. Acta* **29**, 173.

8. Fazakerley, H., Halsall, T. G. and Jones, E. R. H. (1959) *J. Chem. Soc.* 1877.
9. Cerny, J., Vystrcil, A. and Huneck, S. (1963) *Chem. Ber.* 3021.
10. Koops, A. J., Baas, W. J. and Groeneveld, H. W. (1991) *Plant. Sci.* **74**, 185.
11. Blunt, J. W. and Stothers, J. B. (1977) *Org. Magn. Res.* **9**, 439.
12. Andesogan, E. K. and Taylor, E. H. K. (1967) *Chem. Ind. (Lond.)*, 1365.
13. Pyrek, J. St. and Baranowska, E. (1977) *Rocz. Chem.* **51**, 1141.
14. Morikawa, H. and Kitazume, S. (1979) *Ind. Eng. Chem. Prod. Res. Dev.* **18**, 254.
15. Patil, V. D., Nayak, U. R. and Dev, S. (1973) *Tetrahedron* **29**, 341.
16. Benn, W. R. and Dodson, R. M. (1964) *J. Org. Chem.* **29**, 1142.
17. Smith, W. B. (1978) *Ann. Rep. NMR* **8**, 199.
18. Leibfritz, D. and Roberts, J. D. (1973) *J. Am. Chem. Soc.* **95**, 4996.
19. Gaudemer, A., Golfier, M., Mandelbaum, A. and Parthasarathy, R. (1977) *Stereochemistry – Fundamentals and Methods* (Kagan, H., ed.). George Thieme, Stuttgart.
20. Hassner, A. and Heathcock, C. (1964) *J. Org. Chem.* **29**, 1350.
21. Noguchi, S., Nakayama, F. and Morita, K. (1965) *Chem. Pharm. Bull.* **12**, 1180.
22. Inada, A., Kobayashi, M. and Nakanashi, T. (1988) *Chem. Pharm. Bull.* **36**, 609.
23. Kondo, E. and Mitsugi, T. (1975) *Tetrahedron* **29**, 823.
24. Tanaka, R. and Matsunaga, S. (1992) *Phytochemistry* **31**, 3535.
25. Benson, M., Nes, W. D., Nes, W. R. and Landrey, J. R. (1983) *J. Nat. Prod.* **46**, 274.
26. McLean, S., Perpick-Dumont, M., Reynolds, W. F., Jacobs, H. and Lachmansing, S. S. (1987) *Can. J. Chem.* **65**, 2519.
27. Reynolds, W. F., McLean, S., Poplawski, J., Enriquez, R. G., Escobar, L. I. and Leon, I. (1986) *Tetrahedron* **42**, 3419.
28. Schaffner, K., Caglioti, L., Arigoni, D. and Jeger, O. (1958) *Helv. Chim. Acta* **XLI**, 153.
29. Bouvier, P., Berger, Y., Rohmer, M. and Ourisson, G. (1980) *Eur. J. Biochem.* **112**, 549.
30. Bajaj, A. G. and Dev, S. (1982) *Tetrahedron* **38**, 2949.
31. Heftmann, E. (1975) *Phytochemistry* **14**, 891.
32. Gross, E. G. and Leete, E. (1965) *J. Am. Chem. Soc.* **87**, 3479.
33. Heftmann, E. (1973) in *Phytochemistry* Vol. **II**, (Miller, L. P., ed.), pp. 171–266. Van Nostrand Reinhold Company, New York.