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Spectral Assignments and Reference Data

Structural analysis of 3-α-acetyl-20(29)-lupene-24-oic acid, a novel pentacyclic triterpene isolated from the gum resin of *Boswellia serrata*, by NMR spectroscopy

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3α-Acetyl-20(29)-lupene-24-oic acid (1) was isolated from the gum resin of *Boswellia serrata*. Its presence evidently suggests, that the oxidosqualene triterpene pathway of *Boswellia serrata* closely resembles the biosynthetic route already found in other plants. Complete ^{1}H and ^{13}C spectral assignments were derived from 1D and 2D NMR spectra. This is the first compound with the lupene backbone combining a 3α-hydroxy or 3α-acetyl group with the 24-carboxyl group, a configuration which is typical of the classical boswellic acids. Copyright © 2003 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; 2D NMR; triterpene; 3-α-acetyl-20(29)-lupene-24-oic acid; structural assignment

INTRODUCTION

Natural compounds from the family of pentacyclic triterpenes may be pharmacotherapeutically important. Pentacyclic triterpenic acids isolated from extracts of the gum resin of *Boswellia serrata* have shown promising antiinflammatory effects[1–3] and also antitumor[4–6] and antiviral activity.[7] We have recently reported structural data on acetylated and deacetylated α- and β-boswellic acids, including the 3-α-acetyl-9,11-dehydro derivatives of α- and β-boswellic acid.[8] During the isolation of these boswellic acids, we found a novel compound, which we have now identified as 3α-acetyl-20(29)-lupene-24-oic acid (**1**), with the molecular structure shown in Fig. 1.

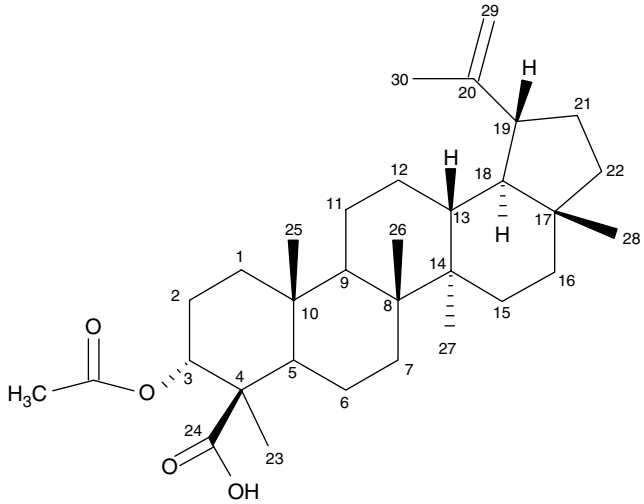

Figure 1. Structure and numbering scheme of 3α-acetyl-20(29)-lupene -24-oic acid (**1**).

EXPERIMENTAL

Samples

Frankincense gum resin of *Boswellia serrata* (Klenk, Schwebheim, Germany) was extracted using the principal methods of Winterstein and Stein.[9] Briefly, pulverized boswellic gum resin was extracted with diethyl ether in a Soxhlet apparatus and the extract was precipitated with $Ba(OH)_2$. The collected solids were acetylated with boiling acetic anhydride and after removal of barium acetate, the mixed anhydrides were cleaved with boiling methanol to yield the 3-acetyl derivatives of the triterpenoic acids. These samples were purified by reversed-phase high-performance liquid chromatography (HPLC) to homogeneity, i.e. ≥99% purity as estimated by HPLC and high performance thin-layer chromatography (HPTLC).[10] UV spectra were recorded during purification with a UVD 340S HPLC photodiode-array detector (Dionex, Idstein, Germany). Mass spectra were recorded with an SSQ 7000 mass spectrometer (Thermo Finnigan, San Jose, CA, USA) in the electron ionization (EI) mode, ionization energy 70 eV, source current 400 μA, temperature programme 30–230 °C at 25 °C min^{-1}, acceleration voltage 1.7 kV.

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3α-Acetyl-20(29)-lupene-24-oic acid **(1)**: white powder upon precipitation with water from methanol; m.p. 234 °C, with decomposition starting at 224 °C. UV, λ_{max} 202 nm, shoulder at 225 nm; EIMS, *m/z* 499(26), 498(82) (M^+ calc. for $C_{32}H_{50}O_4$: 498.75), 484(5), 483(18) ($M^+ - CH_3$), 438(11) ($M^+ - AcOH$), 423(7) ($438^+ - CH_3$), 395(12), 394(31) ($438^+ - CO_2$), 379(7) ($394^+ - CH_3$), 327(10) ($379^+ - C_3H_6$), 283(7), 281(5), 280(15) (M^+ − cleavage of 9–11 and 8–14),[11] 279(6), 271(5), 259(7), 257(8), 255(11), 247(5), 246(13), 234(5), 233(7), 232(9), 231(15), 230(8), 229(21), 221(21), 220(46), 219(50), 218(67) (M^+ − cleavage of 9–11 and 8–14),[11] 217(17), 216(9), 215(12), 213(6), 206(14), 205(35), 204(35) ($218^+ - CH_2$),[11] 189(54) ($204^+ - CH_3$),[11] 55(59) (CH_3CO^+); IR, 3054.76 (w, alkene stretch), 1735.23 and 1695.72 (s, C═O stretch), 1456.42 (m, CH_3 and CH_2 stretch), 1379.63 (m, dimethyl), 1265.19 (ester C–O stretching).

3α-Hydroxy-20(29)-lupene-24-oic acid (**2**) [obtained by alkaline saponification of (**1**)]: EIMS, *m/z* 457(30), 456 (M^+) (100), 442(9), 441(31) ($M^+ - CH_3$), 413(9) ($457^+ - CO_2$), 410(9), 394(6) ($442^+ - CO_2$), 239(15), 238(87) (M^+ − cleavage of 9–11 and 8–14),[11] 219(45), 218(72) (M^+ − cleavage of 9–11 and 8–14),[11] 204(44) ($218^+ - CH_2$),[11] 189(56) ($204^+ - CH_3$).[11]

NMR spectra

Standard conditions were used for the acquisition of the 1D ^{1}H and ^{13}C NMR spectra. ^{1}H and ^{13}C NMR spectra were measured on a Bruker AMX 500 spectrometer operating at 500 and 125 MHz at 300 K. About 15 mg of **1** were dissolved in $CDCl_3$ with TMS as internal standard and transferred to a 5 mm, 400 MHz tube (Kontes, Vineland, USA). DQF-COSY was recorded on a Bruker Avance 400 MHz spectrometer with an FID resolution of 1.724/0.4310 Hz (F_1/F_2), matrix size 512 × 2048 ($F_1 \times F_2$). Other 2D spectra were recorded with $^{1}H-^{1}H$COSY45, HMBC, HMQC, HSQC and HMQC-TOCSY standard pulse sequences. Conditions: $^{1}H-^{1}H$COSY45 FID resolution 3.409/0.562 Hz (F_1/F_2), matrix size 338 × 2048 ($F_1 \times F_2$); HMBC FID resolution 20.35/0.735 Hz (F_1/F_2), matrix size 512 × 4096 ($F_1 \times F_2$); HMQC-TOCSY spin lock period 15 ms, 18.668/1.419 Hz (F_1/F_2), matrix size 512 × 2048 ($F_1 \times F_2$); ROESY spin lock period 300 ms, FID resolution 6.181/1.545 Hz (F_1/F_2), matrix size 512 × 2048 ($F_1 \times F_2$).

Molecular modeling

A structure was modeled employing the InsightII/Discover software (Accelrys, San Diego, CA, USA). The structure sketched according to the NMR restrictions was optimized with the discover-force-field (CVFF91, conjugated gradient) and then run through a molecular dynamics calculation from 0 to 450 K in 10 fs and kept there for

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Table 1. ^{1}H and ^{13}C NMR assignments (δ in ppm, J in Hz) for 3α-acetyl-20(29)-lupene-24-oic acid (**1**)[a]

	^{13}C	^{1}H	HMBC, $J = 2$	HMBC, $J = 3$	HMQC-TOCSY	ROESY	COSY
1′	170,4		2′				
2′	21.36	2.07					
1	34.41	1.07 (d, t, $J_{2\alpha}$ 14.7, $J_{2\beta}$ 5.7)		25	2α, 2β, 3		1β, 2α, 2ε, 25
		1.48					1α, 2α, 2β
2	23.72	2.10			1α, 1β, 3		1α, 1β, 2β, 3β
		1.58					1α, 1β, 2α
3	73.48	5.25 s		23	2α, 2β		2α, 2β
4	46,70		23				
5	50.48	1.37 m		1	6α, 6β, 7[b]		
6	19.53	1.84					6β, 7
		1.62					6α 7
7	34.08	~1.4[b]		26	6α, 6β	15β	6α, 6β
8	40,81		26	27			
9	49.68	1.31 (d, d $J_{11\alpha}$ 10.3, $J_{11\beta}$ 4.5)		25, 26	11α, 11β, 12α, 12β, 13	27	11α, 11β
10	37.66		25				
11	21.08	1.42 (d, t, $J_{11\beta}$ 12.2)			9, 12α, 12β, 13		11β, 12α, 12β, 13α
		1.21 (d, d, d, d)					11α, 12α, 12β
12	25.19	1.06 (d, t, $J_{12\beta}$ 12.6)			9, 11α, 11β, 13, 18	27, 29(z) (w), 30	12β, 11β
		1.68 (d, d, $J_{11\alpha}$ 8.0, $J_{11\beta}$ 6.2)				29(z)	12α, 11α
13	38.02	1.64	18, 19	27	11α, 11β, 12α, 12β, 18, 19		
14	42.92		27, 15α	26			
15	27.43	1.66		27	16α, 16β		15β, 16α, 16β
		1.00 ($J_{16\alpha}$ 4.5)				7β	15α, 16α, 16β
16	35.56	1.36		28	15α, 15β		16β, 15α, 15β
		1.46					16α, 15α, 15β
17	43.03		22α,28				
18	48.25	1.36 (d, t, $J_{19\alpha}$ 11.1)	19	28	12α, 12β, 19, 21α, 21β	29z	19
19	47.95	2.36 (d, d, d, $J_{21\beta}$ 5.8, $J_{21\alpha}$ 11.1)	18	29e, 29z, 30	18, 21α, 21e, 22α, 22β	28, 29(z)	18, 21α, 21β
20	150.92		30,19				
21	29.85	1.90 m ($J_{21\alpha}$ 12)	19		19, 21α, 21β	28	21β, 20, 22,α, 22β
		1.31 m ($J_{22\beta}$ 5.0)				30	21α, 20, 22α, 22β
22	39.98	1.37		28	19, 21α, 21β		22β, 21α, 21β
		1.17					22α, 21α, 21β, 28
23	23.67	1.18					
24	181.26		[c]				
25	13.41	0.76					1α
26	15.98	1.04					
27	14.64	0.97				29(e) (w)	
28	18.01	0.78 m[d]		22α, 22β		19, 29(e) (w)	22β
29	109.33	4, 55(e) m[e]				30 (s)	29(z), 30
		4, 67(z) m				30 (w)	29(e), 30
30	19.30	1.65 m			29(e), 29(z)	19, 21β	29(e), 29(z)

[a] C-8 and C14 are only very weakly distinguishable by the 15α-correlation of C-14, which is supported by comparison with 20(29)-lupeol-3β-acetate[14] and 20(29)-lupenone.[18] Coupling constants in Hz were obtained from DQF-COSY 400 MHz spectra.

[b] No separation between α- and β-peaks.

[c] No cross-peak derived from the carboxyl carbon.

[d] Very weakly resolved multiplet.

[e] Weakly resolved multiplet both for H-29(e) and (z).

100 fs. In addition to the molecular mechanics calculation, the structure was finally minimized with MOPAC/AM1,[12,13] BFGS-optimizer (Broyden–Fletcher–Goldfarb–Shanno pseudo–Newton optimization algorithm), maximum gradient 0.1. The generated PDB files were visualized and printed with Swiss-PdbViewer.[14] Distances were calculated from the model. In the case of swivelling bonds, dihedrals were modified to obtain minimal distances without application of an additional restrained optimization, because the results were only used to illustrate possible ROEs.

RESULTS AND DISCUSSION

Individual carbons were assigned on the basis of the ^{13}C spectrum and with the *J*-resolved ^{13}C spectrum. The two- and three- bond $^{13}C-^{1}H$ cross peaks of the methyl ^{1}H singlets served as the starting point.[15] The 3α-hydroxy orientation appears to correspond to that of the known boswellic acids.[8,16] The pronounced H-1α/H-25 and H-9/C-25 together with the large $J_9-11\alpha = 10.3$ Hz coupling indicates a relaxed chair–chair conformation of both rings A and B. The carboxyl C-24 did not show any cross peaks, but was identified at $\delta = 181.26$ ppm after comparison with our recently published results.[8]

Starting at H-9, the spin system spanning to H-22 via H-11, H-12, H-13, H-18, H-19, H-21 was identified by HMQC-TOCSY. The conformation of the cyclopentyl neighborhood was deduced from the H-19 DQF-COSY cross peaks with H-13, H-18 and H-21. The principal ddd pattern of H-19 is degenerated to a dt with $J_{21\alpha} \approx J_{18} \approx 11.1$ Hz, intensity distribution 1:1:2:2:1:1. The large coupling constants afford a planar orientation of these hydrogens, which is only possible with a transaxial conformation of H-13 and H-18. Left alone with DQF-COSY the dihedral angle between H-18 and H-19 could be around 180° or 0°. The ROEs illustrated in Fig. 2 resolved this question as part of the ROE-led reconstruction above and below the ring plane.

Figure 3 shows a picture of the optimized structure. Calculated dihedral angles from this model structure were H-12α/H-13 = 172.48°, H-13/H-18 = 171.71°, H-18/H-19 = 163.57°, H-19/H-21α = 149.06°, H-19/H-21β = 14.72°, respectively, which is in accordance with the observed coupling constants. Clearly, the last two dihedrals between the cyclopentyl hydrogens would explain a large coupling constant; however only one has been found. This may be caused by the conformational uncertainty of cyclopentyl rings, that was not resolved with these settings of the BFGS-optimizer. The calculated minimal distance from H-29(e) to H-12α is 0.96 Å, which explains the relatively strong ROE. Distances between H-25/H-26, H-27/H-12α were slightly below 2 Å, while the minimal distance of H-27/H-29(e) was 2.04 Å. ROE signals corresponding to these distance calculations were found as listed in Table 1.

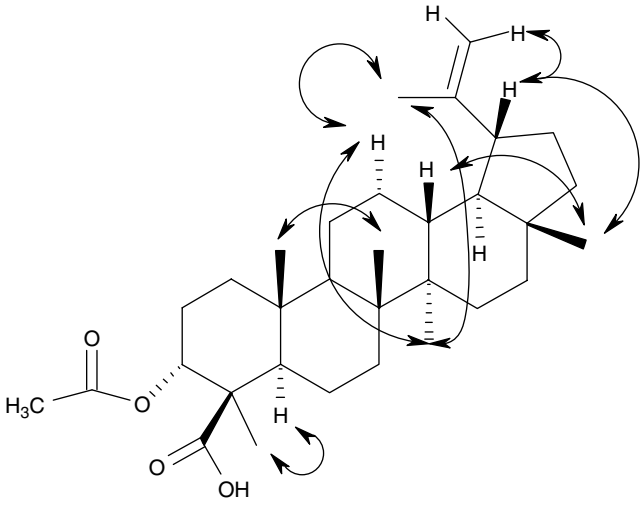

Figure 2. Illustration of the ROEs observed with 3α-acetyl-20(29)-lupene-24-oic acid (**1**), which were important to resolve the conformation. Additional interactions between H-12β/H-30 and H-29(z)/H-18 are also important, but were excluded for clarity.

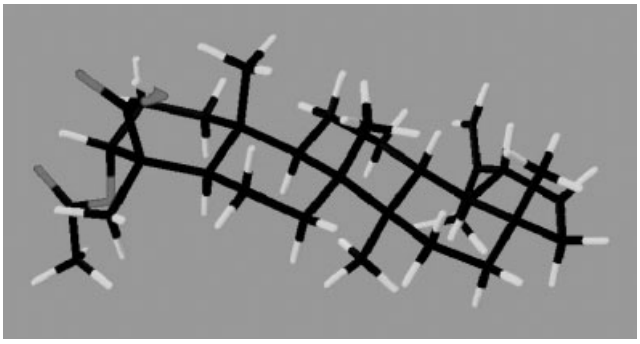

Figure 3. Spatial model in ball and stick representation calculated according to the NMR restraints using the semi-empirical MOPAC/AM1 method. The isopropenyl substituent is directed to the background. All rings are arranged in a nearly perfect plane by *trans*-connections, in contrast to the typical boswellic acids, where rings C and D are *cis* connected.

CONCLUSION

The discovery of 3α-acetyl-20(29)-lupene-24-oic acid (**1**) adds a new member to the range of pentacyclic triterpenic acids found in the gum resin of *Boswellia serrata*. The relative content of **1** in boswellic resin is low, probably because it is a byproduct in the biosynthetic formation of boswellic acids. The respective enzymatic pathways from oxidosqualene to similar pentacyclic triterpenes involve an intermediate lupene cation,[17] which rearranges to the final amyrin, ursol or lupenyl structure preserving the original hydroxyl group orientation. In all known plant triterpenes, except of those isolated from the gum resin of *Boswellia* species, β-orientation of the hydroxy group has been found. The combination of the α-hydroxy and the 24-carboxyl group of this acid and the α- and β-amyrin backbone of the usual lead triterpenes of *Boswellia serrata* may simply indicate low selectivity of the enzymes catalyzing the oxygenation of C-24 for alterations at ring E. This could be verified if the respective 3α-lupeol can be found in the neutral fraction of the resin gum extract.

REFERENCES

1. Ammon H, Mack T, Singh G, Safayhi H. *Planta Med*. 1991; **57**: 203.
2. Sander O, Herborn G, Rau R. *Z. Rheumatol.* 1998; **57**: 11.
3. Gerhardt H, Seifert F, Buvari P, Vogelsang H, Repges R. *Z. Gastroenterol.* 2001; **39**: 11.
4. Hoernlein RF, Orlikowsky Th, Zehrer C, Niethammer D, Sailer ER, Simmet Th, Dannecker GE, Ammon HPT. *J. Pharmacol. Exp. Ther.* 1999; **288**: 613.
5. Heldt MR, Syrovets T, Winking M, Sailer ER, Safayhi H, Ammon HPT, Simmet Th. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1997; **355**(Suppl.): 30.
6. Belsner K, Büchele B, Syrovets T, Simmet Th. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 2001; **363**(Suppl.): 44.
7. Hussein G, Miyashiro H, Nakamura N, Hattori M, Kakiuchi N, Shimotohno K. *Phytother. Res.* 2000; **14**: 510.
8. Belsner K, Büchele B, Werz U, Syrovets T, Simmet Th. *Magn. Reson. Chem.* 2003; **41**: 115.
9. Winterstein A, Stein G. *Hoppe Seyler's Z. Physiol. Chem.* 1932; **208**: 9.
10. Büchele B, Zugmaier W, Simmet Th. *J. Chromatogr. B* 2003; **791**: 21.

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11. Shiojima K, Suzuki H, Kodera N, Kubota K, Tsushima S, Ageta H, Chang HC, Chen YP. *Chem. Pharm. Bull.* 1994; **42**: 2193.
12. Dewar MJS, Zoebisch EG, Healy EF, Stewart JJP. *J. Am. Chem. Soc.* 1985; **107**: 3902.
13. Stewart JJP. *J Comput.-Aided Mol. Des.* 1990; **4**: 1.
14. Guex N, Diemand A, Peitsch MC. *Trends Biochem. Sci.* 1999; **24**: 364.
15. Reynolds WF, McLean S, Burke SJ, Jacobs H. *Magn. Reson. Chem.* 2001; **39**: 757.
16. Schweizer S, Eichele K, Ammon HPT, Safayhi H. *Planta Med*. 2000; **66**: 781.
17. Poralla K. In *Isoprenoids including Carotenoids and Steroids*, vol. 2, Cane DE (ed). Elsevier: Oxford, 1999; 300.
18. Kim E-M, Jung H-R, Min T-J. *Bull. Korean Chem. Soc.* 2001; **22**: 59.