A Further Contribution to the Triterpenoid Constituents of *Glycyrrhiza glabra* L.

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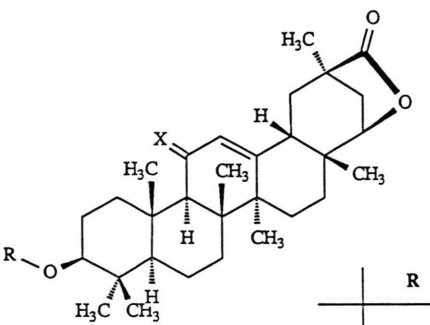
Pentacyclic Triterpenoids, *Glycyrrhiza glabra,* ^{1}H and ^{13}C Chemical Shifts

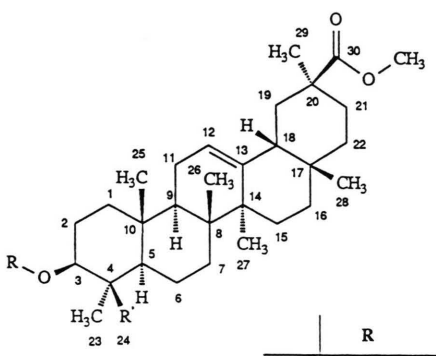
Five pentacyclic triterpenoids have been isolated from the minor constituents of local liquorice roots, one of them (**8**) has not been isolated before from liquorice root. Their structural formulae and stereochemical configuration was determined by spectroscopic methods. ^{13}C and ^{1}H NMR data have been compiled.

Introduction

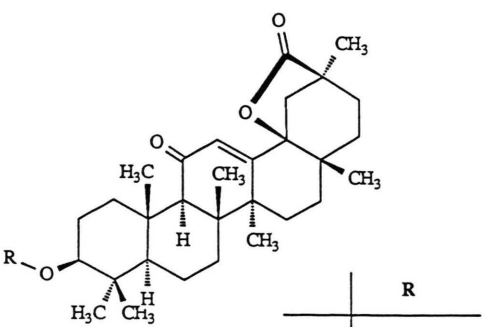
Recent pharmalogical reports have shown that both major and minor constituents of liquorice root (*Glycyrrhiza glabra* L.) form the active principles of many phytotherapeutic drugs in the treatment of gastric ulcers and dermatitis. In addition, they possess anti-inflammatory effects [1]. So it deemed desirable to continue our investigation [2] to identify the nature of the minor triterpenoids existing in the roots of the drug grown in Egypt.



	R	X
4	CH_3CO	H_2
5	H	O
6	CH_3CO	O



	R	R'
1	CH_3CO	CH_3
2	H	CH_2OH
3	CH_3CO	CH_2OCOCH_3



	R
7	H
8	CH_3CO

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Results and Discussion

A successive fractionation on PTLC of the collected mother liquors of methyl glycyrrhetate acetate afforded 25 g of a solid material which was subjected to silica gel column chromatography [2]. Thereby, five triterpenoids which have not been isolated previously from the local drug [2] were obtained. These are 11-desoxoglycyrrhetic acid acetate methyl ester (**1**), 24-acetoxy-11-desoxoglycyrrhetic acid acetate methyl ester (**3**), 11-desoxoglabrolide acetate (**4**), glabrolide acetate (**6**) and a triterpenoid (**8**) which is 3β-acetyl-18β-hydroxy-11-keto-olean-12-en-30-oic acid, 30,18β-lactone. Its saponification product **7** has been synthesized by Canonica *et al.* [3] and **8** by Ogura *et al.* [4]. None of these two compounds, however, has ever been found in a plant. Interestingly, **8** is reported to be a constituent in the marine coelenterate *Echinopera lamellosa* [5].

Although ^{13}C NMR [6] and more recently high-field ^{1}H NMR spectroscopy [7] has proven to be an excellent tool for the structural elucidation of triterpenoids, only very few such data on the compounds discussed here have been published. Thus,

Table I. ^{13}C chemical shifts of the triterpenoids **1**–**6** and **8**[a].

	1	**2**	**3**[b]	**4**	**5**[b]	**6**	**8**
C-1	38.3	39.0	38.4	38.2	39.6	38.6	38.7
C-2	23.5	27.8	22.5	23.5	27.1	23.4	23.4
C-3	80.9	80.8	80.2	80.8	78.7	80.4	80.4
C-4	37.7	42.1	41.0	37.7	41.1	37.9	37.9
C-5	55.2	56.5	55.9	55.3	55.5	54.9	54.8
C-6	18.2	19.1	19.3	18.2	17.9	17.3	17.3
C-7	32.6	33.5	33.0	32.9	33.5	32.9	32.6
C-8	39.8	40.4	39.7	39.5	44.9	44.1	43.8
C-9	47.5	48.4	47.6	47.4	62.4	61.6	61.1
C-10	36.8	37.2	36.7	36.9	37.7	36.9	36.7
C-11	23.5	24.3	23.6	23.3	201.2	199.2	199.2
C-12	122.4	123.1	122.3	125.0	129.9	129.7	122.9
C-13	144.3	145.0	144.3	140.2	166.3	164.1	162.3
C-14	41.5	42.9	41.4	42.1	45.7	44.9	45.5
C-15	26.1	26.7	26.1	26.5	25.4	26.0	26.9
C-16	26.9	27.5	26.9	24.8	24.2	24.9	31.2
C-17	31.9	32.5	31.9	35.7	36.0	35.4	36.3
C-18	48.2	48.4	48.1	44.4	45.3	44.4	87.5
C-19	42.8	43.4	42.8	42.6	39.7	40.7	50.0
C-20	44.2	45.0	44.2	38.5	42.7	41.9	44.4
C-21	31.3	31.8	31.3	38.5	38.6	38.2	32.5
C-22	38.4	39.0	38.3	84.5	84.9	83.7	34.8
C-23	28.2	22.9	28.1	28.0	28.3	27.9	27.9
C-24	16.7	65.0	65.4	16.7	16.0	16.3	16.4
C-25	15.5	16.5	15.4	15.6	16.7	16.6	16.6
C-26	16.8	17.1	16.6	16.8	19.1	18.6	18.5
C-27	25.9	26.3	25.8	24.9	22.7	22.3	22.1
C-28	28.2	28.6	28.2	23.3	24.2	23.8	20.5
C-29	28.5	28.8	28.5	20.3	20.3	20.1	23.0
C-30	177.6	179.1	177.6	180.5	181.2	179.5	179.0
Ac	171.0	–	170.6 171.0	171.0	–	170.9	170.9
	21.3	–	21.1 21.2	21.3	–	21.2	21.3
OCH_3	51.5	51.5	51.5	–	–	–	–

[a] In ppm, relative to the central peak of $CDCl_3$ (δ = 77.0). Solvent: $CDCl_3$ if not otherwise noted. Numbers of protons attached to each carbon have been determined by DEPT experiments and correspond to the structures.

[b] Solvent: $CD_3OD/CDCl_3$ (3:1).

Table II. ^{1}H chemical shifts of the triterpenoids **1, 3, 4, 5, 6** and **8**[a].

	1	**3**[b]	**4**	**5**[b]	**6**	**8**
H-1 α	1.02	1.05	1.08	0.95 (td)	0.99 (td)	0.99 (td)
β	1.02	1.05	1.08	2.67 (dt)	2.71 (dt)	2.70 (dt)
H-2 α	1.60	1.65	1.60	1.6	1.57 (m)	1.6 (m)
β	1.60	1.65	1.60	1.6	1.57 (m)	1.6 (m)
H-3	4.47 (m)	4.55 (m)	4.48 (dd)	3.14 (dd)	4.35 (dd)	4.46 (dd)
H-5	0.83	0.86 (d)	0.82 (d)	0.68 (d)	0.75 (d)	0.75 (d)
H-6 α	1.50	1.63 (m)	?	1.5	1.59 (m)	1.57 (m)
β	1.33	1.33 (m)	?	1.42 (t)	1.41 (t)	1.37 (m)
H-7 α	1.50	1.50 (m)	?	1.63 (td)	1.63 (td)	*ca.*1.6
β	1.30	1.32 (m)	?	1.43 (d)	1.40 (d)	*ca.*1.45
H-9	1.54 (m)	1.58 (m)	1.55 (m)	2.36 (s)	2.30 (s)	2.28 (s)
H-11 α	1.85 (m)	1.85	1.85	–	–	–
β	1.85 (m)	1.85	1.85	–	–	–
H-12	5.23 (m)	5.24 (m)	5.28 (t)	5.54 (s)	5.55 (s)	6.04 (s)
H-15 α	?	?	?	1.28 (m)	1.21 (m)	1.20 (dd)
β	?	?	?	1.88 (m)	1.82 (m)	1.90 (td)
H-16 α	?	?	?	1.88 (m)	1.81 (m)	2.10 (td)
β	?	?	?	1.28 (m)	1.22 (m)	1.40 (m)
H-18	1.85 (m)	1.90 (m)	2.00 (m)	2.15 (dd)	2.16 (dd)	–
H-19 α	?	?	1.73 (t)	1.79 (t)	1.71 (t)	2.55 (d)
β	?	?	1.55 (m)	1.55 (d)	1.55 (d)	1.81 (dd)
H-21 α	?	?	2.18 (d)	2.27 (d)	2.19 (d)	1.70
β	?	?	2.02 (?)	2.08 (ddd)	2.03 (ddd)	[t]
H-22 α	1.25	1.23	4.15 (d)	4.25 (d)	4.18 (d)	[o]
β	1.25	1.23	–	–	–	1.55
H-23	0.85 (s)	0.98 (s)	0.84 (s)	0.97 (s)[c]	0.82 (s)	0.83 (s)
H-24	0.75 (s)	4.33 (d) 4.10 (d)	0.83 (s)	1.09 (s)	0.82 (s)	0.83 (s)
H-25	0.93 (s)	0.94 (s)	0.95 (s)	0.76 (s)	1.10 (s)	1.13 (s)
H-26	0.93 (s)	0.91 (s)	0.92 (s)	1.09 (s)	1.06 (s)	1.14 (s)
H-27	1.10 (s)	1.10 (s)	1.13 (s)	1.39 (s)	1.33 (s)	1.35 (s)
H-28	1.10 (s)	1.10 (s)	0.95 (s)	0.95 (s)[c]	0.95 (s)	1.15 (s)
H-29	0.84 (s)	0.74 (s)	1.14 (s)	1.14 (s)	1.14 (s)	0.92 (s)
Ac	2.02 (s)	2.03 (s) 2.00 (s)	2.03 (s)	–	2.00 (s)	2.01 (s)
OCH_3	3.65 (s)	3.64 (s)	–	–	–	–

[a] In ppm, relative to $CHCl_3$ (δ = 7.24). Solvent: $CDCl_3$. Signal multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet or unidentified multiplicity (due to overlap), δ values without such notation are approximate values; "?": could not be identified safely.
[b] Solvent: $CD_3OD/CDCl_3$ (3:1).
[c] May be interchanged.

we collected the NMR data of **1**–**6** and **8** in the Tables I and II to provide a safer basis for further investigations in this field.

Compound **1** could be identified by comparison of its ^{13}C NMR spectrum with those of closely related derivatives [6].

Compounds **2** (obtained by us by saponification of **3**) and **3** have been isolated from *G. glabra* L. by Canonica *et al.* [8]. Since NMR evidences rely only on the readily identifiable pronounced proton peaks we performed an NOE-difference experiment with **3** to prove that indeed C-24 is oxygenated and not atom C-25: Irradiation of the two diastereotopic and anisochronous H-24 gave rise to a considerable NOE effect at the C-25 proving that the CH_2OR group is in fact on the β-face of the molecule.

Compound **4** (obtained by us by saponification of **5**) and/or **5** have been mentioned in the literature several times. Isolations have been reported from *G. glabra* [9]. The lactone ring formation is detected easily by the absence of ^{1}H and ^{13}C NMR

signals of the methoxy group and the appearance of the typical peaks for a >CH−O-fragment (C-22).

Compound **6** (glabrolide acetate) has been isolated from *G. glabra* by Canonica *et al.* [9]. The ^{13}C NMR spectrum of **6,** isolated from *G. uralensis,* has been published by Chinese authors [10], and their data agree well with ours, except that the signals of C-8/C-20 and C-27/C-28 have to be reversed pairwise.

The previous occurrence of compound **7** (obtained by us by saponification of **8**) and **8** has already been mentioned above. The ^{13}C NMR spectrum not reported previously proves the lactone formation from C-30 to C-18 since a signal corresponding to a quaternary oxygenated carbon is found ($\delta = 87.5$). A comparison with the ^{13}C NMR data of 3-O-acetyl-18β-glycyrrhetic acid methyl ester [5] shows the expected diamagnetic signals shift for C-13 and C-22 due to the γ-position of the carbons with respect to the oxygen at C-18. On the other hand, if the ^{13}C NMR data of **8** and 3-O-acetyl-18α-glycyrrhetic acid methyl ester [6] are compared, many discrepancies rule out that **8** is an α-lactone. *E.g.,* the γ-*gauche* positioned C-22 would experience an 6.4 ppm deshielding although a corresponding shielding effect would be expected. It is emphasized that the ^{13}C signal assignment of **8** is confirmed by two-dimensional homo- and heteronuclear COSY experiments.

The mass spectra of **6** and **8** display characteristic differences which allow the differentiation between them, *i.e.* to determine the lactone orientation. For example, both molecular ions produce fragments with *m/z* 260 but with very different relative stabilities (**6:** 55%, **8:** 14%).

Materials and Methods

^1H and ^{13}C NMR spectra were recorded on a Bruker AM-400 spectrometer (^1H: 400.1 MHz; ^{13}C: 100.6 MHz) using $CDCl_3$ as solvent, IR spectra in nujol with a Beckmann 4220 spectrophotometer, mass spectra on Varian CH-5 and CH-7 at 70 eV, high-resolution mass spectra on a Varian MAT 710 (70 eV), UV spectra on a Shimadzu UV-240. Column chromatography was carried out using silica gel D and monitored by TLC [2].

Liquorice root grown in the mid-western Egyptian desert was extracted and processed as described by Beaton and Spring [11] for the isolation of glycyrrhetic acid acetate. The acetylated mother liquors (70 g) remaining after crystallization of glycyrrhetic acid was methylated. By PTLC fractional separation three zones were obtained, the medium zone of which (25 g) was once again fractionated on a silica gel column leading to the isolation of five triterpenoid aglycones named **1, 3, 4, 6** and **8.** The first fraction of benzene elution gave 50 mg of 11-desoxoglycyrrhetic acid acetate methyl ester (**1**).

Further fractions (from benzene) afforded 60 mg of 24-acetoxy-11-desoxoglycyrrhetic acid acetate methyl ester: (**3**): m.p. 252−3 °C; $[\alpha]_D$ = +172.3 (c = 0.058, $CHCl_3$); UV: 207 nm; IR: 1725, 1250 and 1205 cm^{-1}; MS: *m/z* (rel. intens. %) 570 (M^+, 1.4), 568 (1.8), 511 (1.5), 435 (1.5), 276 (14), 262 (100), 247 (6), 187 (16), 173 (13), 147 (19), 133 (15).

24-Hydroxy-11-desoxoglycyrrhetic acid methyl ester (**2**). 20 mg of **3** were saponified with 3% alcoholic KOH as usual [2] to give 15 mg of **2:** m.p. 280−3 °C; $[\alpha]_D$ = +209 (c = 0.051, $CHCl_3$); MS: *m/z* (rel. intens. %) 486 (M^+, 1.2), 484 (1.9), 427 (1.5), 395 (0.8), 316 (1), 301 (1.3), 276 (14), 262 (100), 247 (2.5), 206 (5), 203 (11), 175 (14), 147 (18), 133 (8).

The next fraction from the benzene elution was 30 mg of 11-desoxoglabrolide acetate (**4**): m.p. 295−7 °C; $[\alpha]_D$ = +53.9 (c = 0.028, $CHCl_3$); UV: 206 nm; IR: 1765, 1725, 1250 cm^{-1}; MS: *m/z* (rel. intens. %) 496 (M^+, 1.7), 494 (1.2), 436 (3.5), 421 (1.5), 393 (1.2), 299 (1.8), 283 (1.4), 246 (100), 203 (3), 190 (52), 175 (12), 147 (13); high-resolution MS: *m/z* 496.3549, calculated for $C_{32}H_{48}O_4$: 496.3553.

The fourth fraction was eluted with benzene/acetone (19:1) to afford 40 mg of glabrolide acetate (**6**): m.p. 327−330 °C (decomp.); $[\alpha]_D$ = +147 (c = 0.037, $CHCl_3$); UV: 248 nm; IR: 1778, 1733, 1660, 1250 cm^{-1}); MS: *m/z* (rel. intens. %): 510 (M^+, 9), 495 (2), 450 (18), 435 (8), 301 (100), 260 (55), 217 (5), 175 (29), 135 (32); high-resolution MS: *m/z* 510.3360, calculated for $C_{32}H_{46}O_5$: 510.3345.

Glabrolide (**5,** 15 mg) was obtained by saponification of **6** (20 mg) with 3% alcoholic KOH. M.p. 360−3 °C; $[\alpha]_D$ = +99 (c = 0.038, $CHCl_3$); MS: *m/z* (rel. intens. %) 468 (M^+, 15), 453 (5), 450 (6), 435

(5), 301 (100), 266 (48), 217 (4), 175 (30), 135 (30); high-resolution MS: *m/z* 468.3245, calculated for $C_{30}H_{44}O_4$: 468.3245.

The last fraction eluted with the above mixture afforded 70 mg of 3β-acetoxy-18β-hydroxy-11-keto-olean-12-en-30-oic acid, 30,18β-lactone (**8**): m.p. 343–5 °C; $[\alpha]_D$ = +269.7 (*c* = 0.067, $CHCl_3$); UV: 245 nm; IR: 1772, 1735, 1660, 1250 cm^{-1}; MS: *m/z* (rel. intens. %): 510 (M^+, 28), 495 (17), 450 (18), 435 (8), 301 (100), 260 (14), 249 (11), 232 (32), 175 (22), 144 (8), 135 (64); high-resolution MS: *m/z* 510.3345, calculated for $C_{32}H_{46}O_5$: 510.3345.

3β,18β-Dihydroxy-11-keto-olean-12-en-30-oic acid, 30,18β-lactone (**7**): 10 mg of **8** was saponified as usual [2] to give 4 mg of **7:** m.p. 329–332 °C (decomp.); $[\alpha]_D$ = +228 (*c* = 0.021, $CHCl_3$); MS: *m/z* (rel. intens. %) 468 (M^+, 31) 453 (28), 450 (5), 435 (9), 301 (100), 260 (16), 255 (15), 232 (38), 175 (30), 135 (66); high-resolution MS: *m/z* 468.3240, calculated for $C_{30}H_{44}O_4$: 468.3245.

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