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GAS CHROMATOGRAPHY-MASS SPECTROMETRY OF OLEANANE- AND URSANE-TYPE TRITERPENES—APPLICATION TO *CHENOPODIUM QUINOA* TRITERPENES

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Key Word Index—*Chenopodium quinoa*; gas chromatography-mass spectrometry; triterpenes.

Abstract—Nine trimethylsilylated pentacyclic triterpenes were separated by GLC on an OV-101 column employing temperature programming. Characteristic retro-Diels–Alder fragmentation was observed in their mass spectra. The fragmentation patterns allowed individual characterization except for certain isomers which, nevertheless, were resolved by GLC, thus permitting their identification. Oleanolic acid and hederagenin were confirmed to be major triterpenes of *Chenopodium quinoa* seed saponins.

INTRODUCTION

Pentacyclic triterpenes of the oleanane and ursane groups are natural components which may be present in plants as saponins [1, 2]. In *Chenopodium quinoa* 'quinoa', triterpene saponins are responsible for the bitter taste of economically important seeds [3]. By TLC [4] and HPLC [5] we have shown that two major triterpenes of quinoa seeds have identical chromatographic mobilities to oleanolic acid and hederagenin. However, as the coupling of HPLC to a mass spectrometer is still at a developmental stage, rapid characterization of the sapogenins present in plant material must at present be achieved by GC-MS.

Recently, one triterpene saponin was isolated from *C. quinoa* and identified as quinoaside A, containing hederagenin [Meyer, B. N., Heinstein, P. F., Burnouf-Radosevich, M., Weisleder, D., Delfel, N. E. and McLaughlin J. L., unpublished results]. This component is found in a mixture with other saponins which have yet to be characterized. Therefore, we have developed a GC-MS method for the resolution and identification of the TMSi ether derivatives of seven oleanane- and two ursane-type triterpenes which are commonly distributed in the form of saponins among higher plants. The separation and identification of *C. quinoa* triterpenes by this GC-MS method is described.

RESULTS AND DISCUSSION

Satisfactory GLC separation of nine silylated pentacyclic triterpenes on OV-101 packing was achieved in 17 min by temperature programming from 265° to 320° at 4°/min. Previous work using isothermal conditions showed poor resolution of derivatized pentacyclic triterpenes [6–11]. Relative retention times (RR_t) were established with respect to cholesteryl acetate (R_t = 9.83 min): β-amyrin (1.15), α-amyrin (1.20), erythrodiol (1.37), oleanolic acid (1.43), ursolic acid (1.49), echinocystic acid (1.53), hederagenin (1.60), gypsogenin (1.67) and queretaroic acid (1.74).

Substituent groups on the TMSi ether derivatives of these triterpenes are given in Table 1. The retention time was influenced by the number and type of functional groups present, and generally increased with increasing molecular weight of the derivatized triterpene. One exception was gypsogenin, with a free aldehyde group, which eluted later than echinocystic acid and hederagenin although it is 74 mass units lower. The position of the second hydroxyl group on an oleanolic acid derivative was also important, thus the three positional isomers, echinocystic acid, hederagenin and queretaroic acid, were all well separated from one another. The ursane compounds, α-amyrin and ursolic acid, were retained longer than their respective oleanane isomers, β-amyrin and oleanolic acid. This may indicate that shifting the methyl group from an axial conformation on C-20 to an equatorial conformation on C-19 increases the planarity of the molecule and, thereby, its retention time. Triterpene isomers usually resolve poorly by chromatography [6, 7]. In our system, α-amyrin separated partially from β-amyrin while ursolic acid and oleanolic acid were well resolved.

The mass fragmentation behaviour of some oleanane and ursane triterpenes has been described previously [12, 13]. In this study, the mass spectral patterns of other triterpenes were investigated. Masses and relative intensities for fragment ions from the silylated triterpenes are listed in Table 2. Triterpenes that contain a Δ^{12}-double bond undergo a retro-Diels–Alder reaction to form fragments containing the ABC*-rings and the C*ED-rings (Scheme 1, C* indicates the presence of only a portion of ring C.). Thus, hederagenin can be distinguished from its isomers, echinocystic acid and queret-

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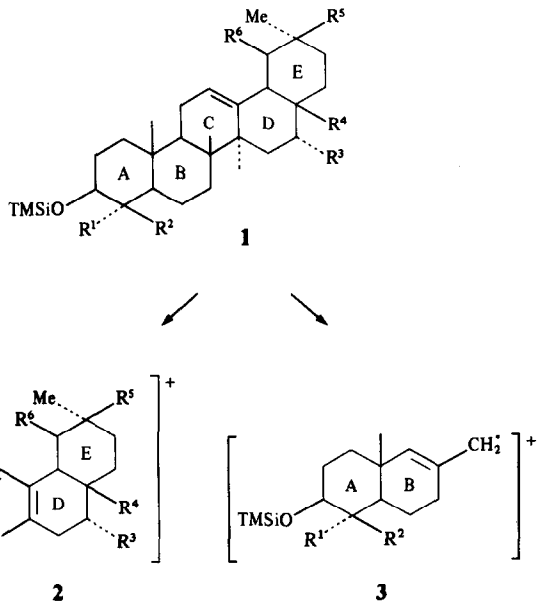
Table 1. Substituent groups on trimethylsilylated triterpenes (1) and their major mass-spectral fragments (2, 3)

Compound	Substituent group					
	R^1	R^2	R^3	R^4	R^5	R^6
β-Amyrin	Me	Me	H	Me	Me	H
α-Amyrin	Me	Me	H	Me	H	Me
Erythrodiol	Me	Me	H	CH_2OTMSi	Me	H
Oleanolic acid	Me	Me	H	COOTMSi	Me	H
Ursolic acid	Me	Me	H	COOTMSi	H	Me
Echinocystic acid	Me	Me	OTMSi	COOTMSi	Me	H
Hederagenin	CH_2OTMSi	Me	H	COOTMSi	Me	H
Gypsogenin	Me	CHO	H	COOTMSi	Me	H
Queretaroic acid	Me	Me	H	COOTMSi	CH_2OTMSi	H



Scheme 1. Mass spectral fragmentation pattern of trimethylsilylated triterpenes.

aroic acid by the formation of the ion with m/z 320 compared with m/z 408 for the latter compounds. The aldehyde group of gypsogenin can be localized in the ABC* portion of the molecule by the ion at m/z 293. In addition, hydroxyl groups on methyl-carbons lose $TMSOCH_2$· and can thereby be distinguished from those on ring-carbons. Hence, erythrodiol, hederagenin and queretaroic acid have an $[M-103]^+$ ion whereas echinocystic acid does not. These differences, together with those in the molecular ion and elsewhere in the spectra, permitted a rapid distinction of all compounds studied except for the pairs α-amyrin–β-amyrin and ursolic acid–oleanolic acid which differ only by the position of a single methyl group. These isomers are, however, resolved by GLC.

The GC-MS method applied to the analysis of triterpenes in quinoa seeds indicated the presence of oleanolic acid and hederagenin with RR_ts of 1.07 and 1.18 (relative to cholesteryl crotonate, R_t = 13.17 min) respectively. The identities of these two triterpenes were determined by comparison of their respective mass spectra to those of standard samples.

The present GLC method for triterpene separation complements the HPLC method since the two provide quite different separations. Such information, together with the distinctive mass spectral fragmentation patterns of the TMSi ether derivatives, should facilitate the characterization and quantitation of triterpenes from natural sources.

EXPERIMENTAL

Plant material. Seeds of *Chenopodium quinoa* Willd., var. Real de Puno were extracted to obtain crude saponins and these were hydrolysed to release the triterpene aglycones as described previously [5].

GLC. A GC apparatus equipped with an FID and a glass column (2 m × 2 mm, packed with 3% OV-101) was used. Temp. was programmed from 265° to 320° at 4°/min. The inlet and the detector temp. were 275° and 330°, respectively. The gas carrier, helium, was set at a flow rate of 30 ml/min. Samples (*ca* 0.2 mg) were dissolved with 20 μl dry pyridine and derivatized by adding 100 μl BSTFA [*N,O*-bis-(trimethylsilyl)trifluoroacetamide] + 1% TMCS (trimethylchlorosilane). TMSi ether derivatives were formed within a few minutes at room temp. Silylation avoids peak tailing and shortens considerably the retention time of triterpenes as compared to methylation and acetylation [7, 8]. Cholesteryl acetate and cholesteryl crotonate were used as internal standards. For plant extracts lacking erythrodiol, cholesteryl crotonate makes a better internal standard than cholesteryl acetate because it elutes close to the compounds of interest.

GC-MS. A Kratos MS 30 gas chromatograph-electron ionization mass spectrometer was fitted with a 1 m × 2 mm glass column packed with 3% OV-1. Temp. programming was from 225° to 300° at 4°/min, and the transfer line was at 275°. Electron impact energy, emission current and ion source temp. of the mass spectrometer were 70 eV, 1.96 mA and 175°, respectively. Detected mass range was extended by operating the instrument on range 2 using a perfluorokerosene standard.

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Table 2. Mass and relative intensity for ions in the electron impact mass spectra of various TMSi pentacyclic triterpenes

Fragment loss*	β-Amyrin	α-Amyrin	Erythrodiol	Oleanolic acid	Ursolic acid	Echinocystic acid	Hederagenin	Gypsogenin	Queretaroic acid
				m/z (relative intensity)					
None	498 (3.1)	498 (6.4)	586 (0.1)	600 (9.0)	600 (5.5)	688 (4.8)	688 (1.6)	614 (11.1)	688 (3.2)
Me·	483 (1.3)	483 (2.2)	571 (1.7)	585 (9.8)	585 (8.4)	673 (2.0)	673 (3.3)	599 (8.4)	673 (4.1)
CO	—	—	—	—	—	—	—	586 (2.3)	—
CHO·	—	—	—	—	—	—	—	585 (1.9)	—
CH_2O	—	—	—	—	—	—	—	584 (1.6)	—
MeCHO	—	—	—	—	—	—	—	570 (2.7)	—
TMSiOH	408 (0.8)	408 (1.5)	496 (94.5)	510 (0.8)	510 (1.2)	598 (11.7)	598 (2.2)	—	598 (3.4)
$TMSiOCH_2$·	—	—	483 (2.4)	—	—	—	585 (1.6)	—	585 (7.4)
TMSiOOCH	—	—	—	482 (23.5)	482 (9.6)	570 (7.3)	570 (12.5)	496 (21.9)	570 (8.4)
TMSiOH + Me·	393 (1.2)	393 (1.6)	481 (4.9)	495 (0.7)	495 (0.6)	—	—	—	—
TMSiOH + TMSiOOC·	—	—	—	393 (1.5)	393 (1.6)	481 (3.2)	481 (1.1)	407 (1.1)	481 (2.1)
TMSiOH + TMSiOOCH	—	—	—	392 (1.0)	392 (0.1)	480 (2.3)	—	—	480 (1.2)
TMSiOH + TMSiOH	—	—	406 (20.0)	—	—	508 (0.9)	508 (0.9)	—	—
ABC*-rings†	218 (100)	218 (100)	306 (0.8)	320 (31.9)	320 (55.2)	408 (1.0)	320 (30.2)	320 (19.1)	408 (1.8)
ABC* + TMSiOH	—	—	216 (100)	—	—	318 (9.7)	—	—	318 (7.7)
ABC* + C28 moiety	203 (19.8)	203 (12.8)	203 (55.2)	203 (100)	203 (70.9)	291 (1.2)	203 (59.6)	203 (71.3)	291 (5.1)
ABC* + C28 moiety + TMSiOH	—	—	—	—	—	201 (23.0)	—	—	201 (28.9)
ABC* + C11 moiety	205 (2.4)	205 (1.8)	293 (1.3)	307 (3.2)	307 (6.2)	305‡ (2.7)	307 (2.2)	307 (8.2)	305‡ (20.3)
C*DE-rings§	279 (3.0)	279 (5.9)	279 (2.6)	279 (5.9)	279 (7.2)	279 (2.2)	—	293 (2.0)	279 (4.4)
[TMSi peak at *m/z* 73]	(14.7)	(34.8)	(60.7)	(91.1)	(100)	(100)	(100)	(100)	(100)

C Represents only a portion of ring C.
†See structure **2**.
‡Additional loss of TMSiOH.
§See structure **3**.

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