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Short communication

Isolation of three triterpenes and several aliphatic alcohols from *Crataegus monogyna* Jacq.

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Abstract

Several aliphatic alcohols and three triterpenes have been isolated from *Crataegus monogyna* Jacq. The aliphatic alcohols isolated were identified as the C_{18} to C_{30} elements of the 1-alkanol homologous series. The structures of triterpene alcohols were established as butyrospermol (3β-lanost 8,24-dien, 3-ol), 24-methylen-24-dihydrolanosterol (24-methylen-5α-lanost-8-en-3β-ol) and cycloartenol (9β,19-cyclo-5α,9β-lanost-24-en-3β-ol).

Keywords: *Crataegus monogyna* Jacq.; Triterpenes; Alcohols; Butyrospermol; 24-Methylen-24-dihydrolanosterol; Cycloartenol

1. Introduction

Several species of *Crataegus* (Rosaceae) have been reported to possess a range of pharmacological properties. One of these species *Crataegus monogyna* Jacq., is found in Península Ibérica, Baleares and Northwest of Africa [1]. *Crataegus monogyna* Jacq. has long been used as a folk medicine due its sedative actions [2], protective effects against arrhythmias [3], and increase of coronary vessel flow [4]. Activities of the extracts on skin microcirculation disturbances have also been studied [5]. Previous chemical studies on *Crataegus monogyna* Jacq. resulted in isolation of know flavonoids [6] and phenolic acids [7]. This study on the hexanoic extracts has now resulted in the isolation of three triterpenic alcohols —butyrospermol, 24-methylen-24-dihydrolanosterol and cycloartenol— and the C_{18} to C_{30} elements of the 1-alkanol homologous series. These compounds have been identified from this species for the first time.

2. Experimental

2.1. General experimental procedures

The MS were recorded at 70 eV on Kratos MS 80 mass spectrometer connected to a NBSLIB2 computer system. The GC was recorded on Chrompack-CP 9000 using helium as carrier gas. GC–MS was performed on a Carlo Erba gas chromatograph linked to a Kratos MS 80 mass spectrometer equiped with a NBSLIB2 data system, using cross-linked 5% phenyl methyl silicone (OV-5, 25 m×0.25 mm×0.23 μm). Samples were run under at programmed temperature

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230°C (6 min) to 300°C at 4°C/min. The trimethyl silyl (TMS) ether derivatives of alcohols were obtained by reaction with pyridine–hexamethyldisilazane–trimethylchlorosilane (9:3:1) at room temperature for 30 min. For analytical TLC silica gel plates (60 F_{254}, 0.2 mm, Merck) were used, and detection was made by spraying with reagent H_2SO_4-CH_3COOH-H_2O (2:40:80) and posterior heating for 5 min (120°C).

2.2. Plant material

Aerial parts of *Crataegus monogyna* Jacq. including twigs, stems and leaves, were collected in Puerto de los Vientos (Serranía de Ronda, Málaga), on February after a cold weather period. A voucher specimen was deposited at the herbarium of the Department of Vegetal Biology (University of Sevilla) (SEV 137261), and was authenticated by Prof. Silvestre.

2.3. Extraction and isolation of triterpenes

The freshly collected plant material (500 g) was extracted with hexane in a soxhlet apparatus. The hexanoic extract was concentrated under reduced pressure using a rotatory evaporator to eliminate organic solvents. 2 g of the residue thus obtained was chromatographed on a silica gel column (60 g, 0.063–0.200 mm and 0.2–0.5 mm, Merck) and successively eluted with solvent portions of increasing polarity of a mixture of hexane and $CHCl_3$, yielding 323 fractions (5 ml). Fractions 120–143 were again chromatographed on a new column of silica gel using a hexane–diethyl ether gradient (200 ml hexane, 100 ml hexane–diethyl ether, 90:10, v/v; 100 ml hexane–diethyl ether, 80:20, v/v and 100 ml hexane–diethyl ether, 70:30, v/v), yielding 114 fractions. Fractions 22–27, corresponding to the hexane–diethyl ether (70:30, v:v) eluate, yielded crystalline fraction (2.8 mg) (TLC silica gel developed with hexane–diethyl ether (70:30, v/v) gave a blue-purple spot with oleum reagent R_F 0.40). Before injection into gas chromatograph, the crystalline fraction was converted to TMS ether derivative by reaction with hexamethyldisilazane and trimethylchlorosilane in pyridine, and the TMS derivatives were separated on a OV-5 capillary column at programmed temperature.

3. Results and discussion

The hexane extract was fractioned twice on silica gel column and a crystalline fraction was isolated. The silyl derivative was analyzed by gas chromatography on capillary column and the GC–MS analyses of the fraction showed the predominance of several aliphatic alcohols and three triterpenoids: butyrospermol, 24-methylen-24- dihydrolanosterol and cycloartenol (Table 1). Additionally, retention indices (I_p) at programmed temperature were calculated for each compound, in relation with those *n*-alkanols of C_n and C_{n+1} [8], and the values obtained are given in Table 1.

Peaks 1 to 13 were TMS ether of a number of aliphatic alcohols (corresponding to C_{18} to C_{30} elements of the 1-alkanol homologous series) which eluted with I_p smaller than 3327. Identification of these peaks was carried out on a comparison of mass spectra with spectral data matches of those in the NBLIB2 library. The TMS ether derivative of the compound corresponding to peak No. 11 with I_p 3195 was not identified.

The MS of TMS ether of the triterpene alcohols corresponding to peak Nos. 14, 15 and 16 (I_p 3366, 3425 and 3508, respectively) showed the following predominant ions:

Peak 14: m/z 498 (M^+, rel. int. 23%), 483 (37%), 393 (72%), 203 (16%), 189 (22%), 145 (35%), 109 (60%), 95 (42%) and 69 (100%).

Peak 15: m/z 498 (M^+, rel. int. 2%), 483 (31%), 408 (96%), 393 (99%), 286 (26%), 189 (26%), 175 (40%), 135 (51%), 109 (53%), 95 (76%), 69 (100%) and 55 (78%). The fragment at m/z 286 involves loss of ring A of 9β,19-cyclopropane sterols [9,10]. In this mechanism the cyclopropyl C-19 is retained with loss of C-6. However, an alternative fragmentation involving retention of C-6 with loss of C-19 to give an ion with the same m/z value [11].

Peak 16: m/z 512 (M^+, rel int. 3%), 497 (14%), 483 (19%), 407 (3%), 393 (47%), 339 (7%), 271 (7%), 189 (17%) and 69 (100%).

The M^+ at 498 ($C_{30}H_{49}OSi$-$(CH_3)_3$) and 512 ($C_{31}H_{51}OSi$-$(CH_3)_3$) indicated that they were a TMS

Table 1

Gas chromatographic retention times and retention indices of TMS ether derivatives of crystalline fraction isolated from *Crataegus monogyna* Jacq.

Peak	Retention time (t_R)[a]	Retention index (I_p)[b]	Relative area (%)	Compound
1	3.05	2178	0.03	Octadecanol-1
2	3.78	2276	0.02	Nonadecanol-1
3	4.47	2351	0.03	Icosanol-1
4	6.87	2552	0.39	Docosanol-1
5	8.02	2630	0.08	Tricosanol-1
6	10.06	2751	2.73	Tetracosanol-1
7	11.44	2826	0.29	Pentacosanol-1
8	13.90	2950	4.61	Hexacosanol-1
9	15.18	3027	0.23	Heptacosanol-1
10	18.10	3148	3.05	Octacosanol-1
11	19.12	3195	0.11	Unidentified
12	20.15	3242	0.40	Nonacosanol-1
13	21.86	3327	2.16	Triacontanol-1
14	22.47	3366	1.29	Butyrospermol
15	23.42	3425	80.87	Cycloartenol
16	24.83	3508	3.56	24-Methylen-24-dihydrolanosterol

[a] Retention time (t_R), defined as time of solute peak maximum minus time of solvent front.

[b] Retention indice (I_p), estimated according to Van den Dool and Kratz [8].

ether of C_{30} triterpene alcohols. Spectral data corresponding to those of butyrospermol, cycloartenol and 24-methylen-24-dihydrolanosterol reported in the literature [12–15].

Their percentage distribution varied considerably. The major constituent of this mixture was cycloartenol. Cycloartenol was the compound corresponding to peak No. 15 and accounted 80.87% of the fraction (equivalent to 113.22 mg of cycloartenol by 100 g of plant). This compound was accompanied by other triterpene alcohols: butyrospermol and 24-methylen-24-dihydrolanosterol, that were present in minor quantities 1.29% and 3.56%, respectively (equivalent to 1.80 and 4.98 mg/100 g of plants, respectively). Aliphatic alcohols accounted 14.02% of the mixture, equivalent to 19.63 mg/100 g plant.

Butyrospermol, 24-methylen-24-dihydrolanosterol and cycloartenol, and C_{18} to C_{30} aliphatic alcohols have been described in *Crataegus monogyna* Jacq. for the first time.

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