Influence of the Parasite *Viscum cruciatum* Sieber on the Chemical Constituents of *Crataegus monogyna* Jacq.

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A phytochemical study of two plant species, *Viscum cruciatum* Sieber and *Crataegus monogyna* Jacq., was completed to investigate the influence of the parasite *Viscum cruciatum* on the host *Crataegus monogyna*. The study was carried out with two samples and consisted of hexane extracts of the *Viscum cruciatum* parasitizing on *Crataegus monogyna* and *C. monogyna*. In these samples urso acid, \(\beta\)-sitosterol and a triterpene fraction were found that contained mainly butyrospermol (3\(\beta\)-lanost 8, 24-dien, 3-ol), 24-methylene-24-dihydrolanosterol (24-methylene-5\(a\)-lanost-8-en-3-ol), cycloartenol (9\(\beta\), 19-cyclo-5\(a\), 9\(\beta\)-lanost-24-en-3-ol), \(\beta\)-amyrin (olean-12-en-3\(\beta\)-ol) and several aliphatic alcohols identified as the C\textsubscript{18} to C\textsubscript{30} members of the 1-alkanol homologous series.

\(\beta\)-Amyrin acetate was only isolated from *Viscum cruciatum* and was not found in *Crataegus monogyna*.

Introduction

*Viscum cruciatum* Sieber (Viscaceae) parasitizes a variety of hosts in the circummediterranean area (Ahumada et al., 1995; Ayuso et al., 1985; Ayuso et al., 1987; Ayuso et al., 1988). The plant has been evaluated for cytostatic activity, *Crataegus monogyna* Jacq. (Rosaceae) is a small thorny tree that has been previously investigated (Garcia et al., 1987; Ayuso et al., 1985; Ayuso et al., 1986), protective effects against arrhythmias (Costa et al., 1986) and increase of coronary vessel flow. In our laboratory, the composition of the hexane extracts of *Crataegus monogyna* Jacq. has been previously investigated (Garcia et al., 1997). In this paper we studied the composition of hexane extracts of *C. monogyna* Jacq. parasitized with *Viscum cruciatum* Sieber and compared these components to those the hexane extract of the parasite, resulted in the isolation of three triterpenic alcohols, butyrospermol, 24-methylene-24-dihydrolanosterol and cycloartenol, and the C\textsubscript{18} to C\textsubscript{30} members of the 1-alkanol homologous series. The triterpene, \(\beta\)-amyrin acetate was only isolated from *Viscum cruciatum* Sieber.

Material and Methods

General experimental procedure

The MS were recorded at 70 eV on a Kratos MS 80 mass spectrometer. The GC operated with a Chrompack-CP 9000 using helium as the carrier gas. GC-MS was performed on a Carlo Erba gas chromatograph linked to a Kratos MS 80 mass spectrometer equipped with a NBSLIB2 data system, using cross-linked 5% phenyl methyl silicone (OV-5, 25 m x 0.25 mm x 0.23 \(\mu\)m). Samples were run under at programmed temperatures 230°C (6 min) to 300°C at 4°C/min. The trimethylsilyl (TMS) ether derivatives of alcohols were obtained by reaction with a mixture pyridine/hexamethyldisilazane/trimethylchlorosilane (9:3:1 v/v/v) and subsequent heating for 5 min (120°C).

Plant material

Aerial parts of the parasite *Viscum cruciatum* Sieber and the host *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 of each species was deposited at the herbarium of the Department of Plant Biology (University of Sevilla) (SEV-F and SEV 137261, respectively), and were authenticated by Prof. S. Silvestre.

**Extraction and isolation of triterpenes**

Plant material (500 g) of each sample was extracted with hexane in a soxhlet apparatus. From the hexane extracts of *Viscum cruciatum* V and *Crataegus monogyna* C we obtained a white amorphous powder (0.29% V1 and 0.05% C1, respectively) by precipitation in the cold (5–10 °C), (TLC silicagel developed with n-hexane/diethyl ether (70:30) gave a purple spot with oleum reagent Rf 0.09). The hexane extracts were concentrated under reduced pressure using a rotary evaporator to eliminate organic solvents. Residues of each sample (2 g) were then chromatographed on a silica gel column (60 g, 0.063–0.200 mm and 0.2–0.5 mm, Merck) and successively eluted with n-hexane/CHCl3 (90:10, 80:20, 70:30, 50:50, 30:70 and 10:90 v/v) yielding 169 fractions and 436 fractions respectively (5 ml each).

Fractions 44–67, corresponding to the n-hexane/CHCl3 80:20 v/v eluate, from hexane extracts of *Viscum cruciatum*, yielded a compound V2 (TLC silica gel developed with n-hexane/diethyl ether (70:30 v/v) gave a orange-purple spot with oleum reagent, Rf (0.92) by preparative chromatography on silica gel.

Fraction 76–106 of the column from extract of parasitised *Crataegus monogyna* were again chromatographed on a new column of silica gel using an n-hexane/diethyl ether gradient (n-hexane, n-hexane/diethyl ether 90:10, 80:20, 70:30, 60:40, and 50:50 v/v), yielding 139 and 105 fractions, respectively.

From fractions 11 to 34 and 35 to 83, from the second column of *Viscum cruciatum* and 151–214 of the column from extract of parasitised *Crataegus monogyna* were again chromatographed on a new column of silica gel using an n-hexane/diethyl ether gradient (n-hexane, n-hexane/diethyl ether 90:10, 80:20, 70:30, 60:40, and 50:50 v/v), yielding 139 and 105 fractions, respectively.

From fractions 11 to 34 and 35 to 83, from the second column of *Viscum cruciatum* the fraction V3 and compound V4 were isolated by preparative chromatography on silicagel.

From fractions 8 to 12, from the second column of *Crataegus monogyna*, and from the n-hexane/diethyl ether (70:30) eluate, a crystalline fraction (0.09%) C2 was obtained, and fractions 24–27 from the n-hexane/diethyl ether (60:40) yielded compound C3 by preparative chromatography on silicagel.

V3 and C2 silica gel TLC developed with n-hexane/diethyl ether (70:30), gave a blue-purple spot with oleum reagent (Rf = 0.40). Before injection into the gas chromatograph, the fractions were converted to TMS ether derivatives by reaction with hexamethyldisilazane and trimethylchlorosilane in pyridine, and the TMS derivatives were separated on a OV-5 capillary column at programmed temperature and the GC-MS analysed.

**Results and Discussion**

Ursolic acid (V1, C1): mp 271–272 °C; UV(C13CH) λmax 235, 285 nm; EIMS m/z (rel.int.%) (M+) 456(6), 411(2), 300(6), 248(100), 219(8), 203(44), 133(33), 119(19).

β-amyrin acetate (V2): mp 235–237 °C; UV(C13CH) λmax 236 nm; EIMS m/z (rel.int.%) (M+) 468(3), 408(3), 218(100), 203(70), 189(40).

β-sitosterol (V4, C3): mp 140–141 °C; UV(C13CH)λmax 236, 262 nm; EIMS m/z (rel.int.%) (M+) 414(25), 381(11), 145(36), 95(50), 81(68), 69(52), 55(86), 43(100).

Fraction V3: The trimethyl silyl derivative of fraction V3, was analyzed by gas chromatography on a capillary column and GC-MS analyses of the fraction showed a predominance of several aliphatic alcohols (74.41%) and four triterpenoids: β-amyrin, butyrospermol, 24-methylene-24-dihydro-lanosterol and cycloartenol. Additionally, retention indices (Ip) at programmed temperature were calculated for each compound, in relation with those n-alkanes of Cn and Cn+1 (Dabrio, 1971), and the values obtained are given in Table I.

Peaks 1 to 13 were TMS ethers of a number of aliphatic alcohols (from C18 to C30 members of the 1-alkanol homologous series) that eluted with Ip less than 3328.

Identification of these peaks was carried out by comparison of mass spectra with spectral data in the NBSLIB2 library. The TMS ether derivative of the compound corresponding to peak No. 11 with Ip 3197 was not identified. The MS of the TMS ether of the triterpene alcohols corresponding to peaks No. 14, 15, 16 and 17 (Ip 3340, 3372, 3460 and 3570 respectively) showed the following predominant ions:
The major constituents of this mixture were the aliphatic alcohols (74.4%); however, the triterpene alcohols were present in minor quantities. Butyrospermol (13.8%), cycloartenol (0.2%) and 24-methylene-24-dihydrolanosterol (7.6%) also were present.

The fraction C2: The silyl derivative was analyzed by gas chromatography on a capillary column and the GC-MS analyses of the fraction showed the presence of several aliphatic alcohols (9.2%) and fourth triterpenoids: β-amyrin, butyrospermol, 24-methylene-24-dihydrolanosterol and cycloartenol. Retention index (Ip), retention time (tR) and relative area (%) are given in Table II.

The major constituent of this mixture was cycloartenol. Cycloartenol was the compound corresponding to peak no.16 and accounted for 79.5% of the fraction (equivalent to 67.0 mg of cycloartenol for 100 g of plant material). This compound was accompanied by other triterpene alcohols: butyrospermol (7.8%), 24-methylene-24-dihydrolanosterol (2.7%) and β-amyrin (0.5%), (equivalent to 7.0, 2.4 and 0.4 mg/100 g of plant, respectively). Aliphatic alcohols accounted for 9.2% of the mixture, equivalent to 8.3 mg/100 g plant material.
Cycloartenol, butyrospermol, 24-methylene-24-dihydrolanosterol and the aliphatic alcohols have been described in *Crataegus monogyna* Jacq. (Garcia et al., 1996), but these compounds are identified from *Viscum cruciatum* Sieber for the first time.

β-Amyrin has not been previously detected in *Crataegus monogyna* Jacq. For this reason, we conclude that this compound, when *Crataegus* is parasitized by *Viscum cruciatum* Sieber, can be synthesized by the parasite.