ACKNOWLEDGMENTS

This work was supported by DIEXAT (Universidad de Antofagasta), Grant B-003, and FONDECYT, Grant 1053-84. The authors thank Professor C. Marticorena, University of Concepcion, for the identification of the plant material, Professor K. Naya, Kwansei Gakuin University, for kindly supplying a specimen of 6β-hydroxyeremophilanolide and 6β, 8β-dihydroxyeremophilanolide, and Professor K. Yamada, Nagoya University for his generous gift of senkirkine. We are indebted to Professor W.A. Ayer, Alberta University, for hrms measurements and to Professor F. Bohlmann, Technical University of Berlin, for running 400 MHz ^{1}H-nmr spectra.

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Received 25 November 1985

STARFISH SAPONINS, PART 28.[1] STEROIDAL GLYCOSIDES FROM PACIFIC STARFISHES OF THE GENUS *NARDOA*[2]

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Continuing our work on biologically active compounds from starfish (1), we have been working on glycosides of the Pacific starfishes *Nardoa novaecaledonia* Perrier and *Nardoa gomophia* Gray and have isolated several known steroidal glycosides previously found in *Halityle regularis* (2). From *N. gomophia* we have also isolated the sulfated glycosides, marthasteroside A_1 (3), thornasteroside A (4), and halityloside I (2), and two polyhydroxysteroids (25*S*)-5α-cholestane-3β,6α, 8,15β,16β,26-hexol (2), and (24*S*)-5α-cholestane-3β,6α,8,15β,24-pentol (5), all known compounds described from starfish. Table 1 summarizes the results.

EXPERIMENTAL

ANIMAL COLLECTION AND EXTRACTION.—*N. novaecaledonia* was collected in October 1983, and *N. gomphia* in March 1984, off Nouméa, New Caledonia, and identified by Mr. Michel Jangoux of the Université Libre de Bruxelles. Samples of each species are deposited in the Centre ORSTOM, Nouméa. The starfish were chopped and soaked with H_2O for 4 h. The aqueous extracts were centrifuged and passed through a column of Amberlite XAD-2 (500 g for each column), which was washed with H_2O (one bed volume) and then with MeOH. The MeOH eluates were taken to dryness to give 2.9 g of glassy material from *N. novaecaledonia* and 4.7 g from *N. gomophia*.

[1]For Part 27, see C. Pizza, L. Minale, D. Laurent, and J.L. Menou, *Gazz. Chim. Ital.*, **115**, (1985).

[2]This contribution is part of the Progetto Finalizzato "Chimica Fine e Secondaria" CNR, Rome, Italy.

ISOLATION OF GLYCOSIDES AND POLAR STEROIDS.—For *N. novaecaledonia*, the MeOH eluate was chromatoghraphed on Sephadex LH-60 (4×80 cm, 100 g) with MeOH-H_2O (2:1) (flow rate 13 ml/h). Fractions (9 ml) were collected; fractions 50-60 contained 350 mg of a complex mixture of sulfated material (not further investigated); fractions 61-130 contained steroidal glycosides (960 mg) and other compounds, and fractions 131-160 contained a mixture of tryptophan and tryptamine (85 mg), identified by comparison (tlc and ^{1}H nmr) with authentic compounds. The glycoside fraction was further chromatographed on Sephadex LH-20 (2×60 cm; eluent, MeOH; flow rate 32 ml/h; fractions of 8 ml were collected) to give 256 mg of partially purified glycosides (fractions 53-65), which were submitted to droplet countercurrent chromatography [dccc; $CHCl_3$-MeOH-H_2O (7:13:8) in the ascending mode at a flow of 25 ml/h; fractions of 5 ml were collected].

Fractions 55-75 contained halityloside A; fractions 80-103 contained halityloside B and fractions 122-150 contained halityloside D. Each of the above fractions was further purified by hplc on a μ-bondapak C-18 column (7.8 mm × 30 cm) using 25% H_2O in MeOH. Quantities isolated are shown in Table 1.

TABLE 1. Compounds Isolated from *Nardoa* Species

Compound	[α]D	fabms (M+Na)	*Nardoa novaecaledonia*[a] (mg)	*Nardoa gomophia*[b] (mg)	Reference
Halityloside A	−3.0°	813	18	15	(2)
Halityloside B	−5.0°	797	13	15	(2)
Halityloside D	−13.0°	783	14	5	(2)
Halityloside E	−20.4°	767	—	30	(2)
Halityloside H	−4.7°	827	—	5	(2)
Marthasteroside A_1	+3.3°	1435	—	19	(3)
Thornasteroside A	+5.3°	1289	—	12	(4)
Halityloside I	—	883	—	7	(2)
(24*S*)-5α-cholestane-3β,6α,8,15β,24-pentol	+12.5°	—	—	15	(5)
(25*S*)-5α-cholestane-3β,6α,8,15β,16β,26-hexol	±0°	—	—	5	(2)

[a]From 6 kg fresh material.
[b]From 5.8 kg fresh material.

For *N. gomophia*, a similar isolation procedure was used. Chromatography of the MeOH eluate on Sephadex LH-60 gave two main fractions. The first fractions (25-45) contained the crude sulfated "asterosaponins" (650 mg), which were submitted to dccc [*n*-BuOH-Me_2CO-H_2O (3:1:5) in the descending mode at a flow of 18 ml/h; fractions of 7 ml were collected] to give, in fractions 92-119, 50 mg of a mixture of marthasteroside A_1 and thornasteroside A, which were finally separated by hplc on a μ-bondapak C-18 using 55% H_2O in MeOH. The successive fractions (46-87) (1.9 g) contained a mixture of steroidal glycosides, polyhydroxysteroids, halityloside I, and other materials. These fractions were submitted to dccc [$CHCl_3$-MeOH-H_2O (7:13:8) in the descending mode; flow rate 18 ml/h; fractions of 3 ml were collected] and afforded in the more polar fractions (211-258) halityloside I, which was further purified by hplc on a μ-bondapak C-18 column with 45% H_2O in MeOH. The less polar fractions (23-162, 346 mg) were further submitted to dccc with the same solvent system as above but in the ascending mode (fractions of 5 ml were collected) and afforded fractions, which were then submitted to hplc on a μ-bondapak C-18 column with 25% H_2O in MeOH to give pure compounds.

IDENTIFICATIONS.—Identification of each compound was based on hplc, optical rotations, ^{1}H nmr and fabms and comparison with authentic samples. In the case of halitylosides A, B, D, and E, the ^{13}C-nmr spectra were also determined.

Full details of the identifications are available on request to the senior author.

ACKNOWLEDGMENTS

We thank the divers G. Bargibant, J.L. Menou, and P. Tirard of the Centre ORSTOM de Nouméa for collection of the animals (*N. gomophia*). We also thank Miss D. Mascagna for her help in the isolation work.

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Received 13 December 1985

CHEMICAL COMPOSITION OF *SANTOLINA CHAMAECYPARISSUS* SSP. *SQUARROSA* ESSENTIAL OIL

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Santolina chamaecyparissus L. ssp. *squarrosa* DC. (Asteraceae) grows in eastern Spain. This plant is an aromatic dwarf shrub commonly known as "Manzanilla de Mahón" or "Abrótano hembra." The flower of this plant is used in folk medicine because of its antispasmodic, digestive, antiinflamatory, antiseptic, and antimicrobial properties (1).

Several studies concerning the composition of the essential oil from the species have been reported, but none have been about the essential oil from *squarrosa* ssp., which is endemic in the western Mediterranean area. This paper reports on the chemical composition of *S. chamaecyparissus* ssp. *squarrosa* essential oil.

EXPERIMENTAL

PLANT MATERIAL.—Fresh leaves and flowers of *S. chamaecyparissus* ssp. *squarrosa* were collected in Ayora-Enguera (Valencia) in June 1983. A voucher specimen has been deposited at the Department of Botany, Faculty of Pharmacy, University of Valencia.

EXTRACTION OF ESSENTIAL OIL.—Fresh plant material was subjected to steam distillation for 2.5 h using a modified Clevenger apparatus which yielded a yellowish essential oil (0.4% v/w).

LIQUID SOLID CHROMATOGRAPHY (lsc).—The oil was fractionated using lsc on a column (3.5×75 cm) of deactivated silica gel Merck (70-230 mesh ASTM) by addition of 5% H_2O followed by gradient elution with hexane, mixtures of hexane/CH_2Cl_2, and CH_2Cl_2 in order to separate the hydrocarbons and the oxygenated components of the oil.

GAS-LIQUID CHROMATOGRAPHY (glc).—The oil and its fractions obtained by lsc were analyzed by glc. Glc was performed using a Hewlett-Packard 5830 A gas chromatograph, equipped with FID, coupled to a 18850 A H-P data integrator. Conditions were as follows: High performance capillary column 5% OV-17 (25 m×0.20 mm) programmed from 80-150° (rate 4°/min); the carrier gas was nitrogen (split technique ratio 1:100); injection 225°; FID 250°.

GAS CHROMATOGRAPHY-MASS SPECTROMETRY (gc/ms).—Gc/ms was performed using an H-P 5995 gas chromatograph mass spectrometer with a membrane separator coupled to an H-P 9825 B control system. Conditions were as follows: High performance capillary 5% OV-17 (25 m×0.20 mm) was used in the same conditions as reported above for glc analyses; the carrier gas was helium (split technique ratio ca. 1:100); electron energy 70 eV; ion source temperature 150°.

Glc analysis showed the presence of 50 components: 39 compounds were identified, of which 28 corresponded to the monoterpenic fraction and 11 to the sesquiterpenic fraction. The essential oil contained a higher proportion of oxygenated compounds than hydrocarbons. Camphor (25.19%), *p*-cymene, 1,8-cineole, bornyl and isobornyl acetate, *allo*-aromadendrene, and α-muurolene are the main constituents, totalling 68.11% of the essential oil. Two sesquiterpene alcohols were detected ($M^+=222$ and $M^+=220$), but they have not been identified.

Identified compounds and their peak area percentages are listed in Table 1 according to their order of elution.