324. *Chemical Investigation of* Cynara scolymus, *L. Part I. The Steroids of the Receptacles and Leaves.*

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Investigation of the receptacles of the Egyptian variety of artichoke led to the isolation of taraxasterol, ψ-taraxasterol, stigmasterol, β-sitosterol, and a new trihydroxy-steroid sapogenin, cynarogenin. These constituents have also been isolated from artichoke leaves together with a wax composed of an ester and a hydrocarbon.

Cynara scolymus, L. (Compositæ) is the commonly cultivated artichoke of Egypt. After earlier investigations [1] of artichoke varieties Fruhstorfer [2] isolated taraxasterol and ψ-taraxasterol from the nonsaponifiable matter of an unspecified alcoholic extract, Suchý, Herout, and Sorm [3] reported a bitter principle, cynaropicrin, from artichoke leaves.

From a light-petroleum extract of the air-dried receptacles of the Egyptian variety of artichoke we have obtained free taraxasterol. Further, from the unsaponifiable fraction of this extract we isolated taraxasterol and impure ψ-taraxasterol. The former gave pure esters but ψ-taraxasterol could not be obtained pure except by treatment with formic acid and deformylation.[4] Formic acid isomerises traces of taraxasterol which contaminate the ψ-isomer, since the former could be converted into ψ-taraxasteryl formate by this reagent.

When a benzene solution of the non-crystalline portion of the nonsaponifiable material was chromatographed on alumina, a mixed polyterpene passed through whereas a crystalline phytosterol mixture was adsorbed, but eluted with boiling ethanol. This phytosterol could not be fractionated by Windaus's method,[5] but its 3,5-dinitrobenzoate could be separated into stigmasteryl 3,5-dinitrobenzoate,[6] and β-sitosteryl 3,5-dinitrobenzoate.[7] The positions of the two double bonds in stigmasteryl acetate were established by infrared spectra. The absorptions at 975 cm.$^{-1}$, and at 840 and 800 cm.$^{-1}$, indicate a Δ^{22}- and Δ^{5}-bond, respectively.[8]

A light-petroleum extract of the leaves similarly gave taraxasterol and ψ-taraxasterol and the same phytosterol mixture as before.

The light-petroleum extract also gave an alcohol-insoluble wax, separated into an ether-insoluble wax A and an ether-soluble wax B. Wax A, $C_{44}H_{88}O_2$ or $C_{46}H_{92}O_2$, appears to contain an ester group; on the other hand, wax B appears to be a paraffin $C_{32}H_{66}$—$C_{34}H_{70}$.

When the concentrated alcoholic extract of the defatted receptacles was freed from water-soluble components and fractionated, a steroidal sapogenin, m. p. 305° (decomp.), now named cynarogenin, was isolated. Analysis of cynarogenin and its derivatives indicated the formula $C_{27}H_{41}O_2(OH)_3$. It gave the general steroid colour reactions but its m. p. and that of its acetate are different from those of the well-characterised trihydroxy-sapogenins, digitogenin,[9] agavogenin,[10] nologenin,[11] and agapanthagenin.[12]

[1] Roffo, *Bol. Inst. med. expil. Estud. Cancer*, 1943, **20**, 65; Neto, Gandra, and Ribeiro, *Rev. Faculdade med. vet., Univ. Sao Paulo*, 1943, **2**, 111; Panizzi and Scarpati, *Nature*, 1954, **174**, 1062; *Gazzetta*, 1954, **84**, 792; Panizzi, Scarpati, and Scarpati, *Gazzetta*, 1954, **84**, 806.
[2] Fruhstorfer, *Chem. Ber.*, 1954, **87**, 423.
[3] Suchý, Herout, and Sorm, *Coll. Czech. Chem. Comm.*, 1960, **25**, 507.
[4] Burrows and Simpson, *J.*, 1938, 2042; Morice and Simpson, *J.*, 1940, 795; 1941, 181.
[5] Windaus and Hauth, *Ber.*, 1906, **39**, 4378.
[6] Campbell, Shepherd, Johnson, and Ott, *J. Amer. Chem. Soc.*, 1957, **79**, 1127.
[7] Soliman and Saleh, *J.*, 1954, 1506.
[8] Beher, Parsons, and Baker, *Analyt. Chem.*, 1957, **29**, 1147.
[9] Tschesche, *Ber.*, 1935, **68**, 1090.
[10] Marker, Wagner, Ulshafer, Wittbecker, Goldsmith, and Ruof, *J. Amer. Chem. Soc.*, 1943, **65**, 1199.
[11] Nishikawa, Morita, Hagiwara, and Inoue, *J. Pharm. Soc. Japan*, 1954, **74**, 1165.
[12] Stephen, *J.*, 1956, 1167.

The infrared spectra of cynarogenin triacetate in carbon disulphide have bands at 1739s, 1370s, 1242s, 1033s, 980s, 960m, 920w, 900m, and 840m cm.$^{-1}$.

From present knowledge,[13] cynarogenin appears to be a steroid *iso*sapogenin having two of its hydroxyl groups in the 2- and the 3-position. The band at 1739 cm.$^{-1}$ is associated with the acetate-carbonyl absorption, the 1370 cm.$^{-1}$ band is due to the methyl and methylene bending vibrations, and the bands at 1242, 1033, and 960 cm.$^{-1}$ are attributed to the presence of C_2 and C_3 acetyl groups. On the other hand, the side-chain is associated with a strong band superimposed on that at 1033 cm.$^{-1}$ besides the bands at 980, 920, 900, and 840 cm.$^{-1}$. As the 900 cm.$^{-1}$ band is stronger than the 920 cm.$^{-1}$ band the "*iso*" configuration of ring F is indicated.[14]

Cynarogenin was also obtained from alcoholic extracts of the defatted leaves.

In an attempt to isolate cynarine from the water-soluble fraction of this extract by the lead-complex method,[1] a brown phenolic deposit was obtained. This afforded traces of cynarine and a yellowish substance similar to cynarine in properties.

Experimental

The light petroleum used had b. p. 55—65°. The solvent for optical rotations was chloroform. Microanalyses were by Herr Alfred Bernhardt, Mulheim, Ruhr.

Extraction of the Receptacles with Light Petroleum.—The air-dried and crushed receptacles of *Cynara scolymus*, L. (5 kg.) were extracted (Soxhlet) with light petroleum. The extract yielded, on distillation, a greenish residue (190 g.) which solidified on cooling. It was dissolved in warm light petroleum (400 ml.) and cooled in ice for several hours, after which time a greenish-white solid (15 g.), m. p. 145—155°, separated; the filtrate yielded a viscous residue on distillation. The solid was digested with light petroleum, and the insoluble portion was repeatedly crystallised from ethanol until its m. p. was 215—217°, and was not depressed on admixture with taraxasterol. Its acetate crystallised from ethyl acetate as plates, m. p. and mixed m. p. 248—250°.

The Nonsaponifiable Material.—The viscous oily residue (100 g.) was refluxed with 10% ethanolic potassium hydroxide (500 ml.) for 2 hr. during which the sterol separated in lumps. The mixture was diluted with water and extracted with ether from which the nonsaponifiable material (50 g.) was recovered as a sticky solid. On acidification of the soapy solution and extraction with ether, greenish solid fatty acids (30 g.) were obtained.

Purification of the Sterol.—The nonsaponifiable material (50 g.) was washed with ice-cold ether until the sterol was obtained as a white solid (35 g.), m. p. 150—155°. It crystallised from light petroleum (charcoal), and then from ethanol, as needles, m. p. 190—195°. The noncrystallisable residue recovered from the ethereal washings and from the mother liquor was investigated as described below.

Isolation of Taraxasterol.—The foregoing sterol (6 g.) was heated in pyridine (20 ml.) and acetic anhydride (20 ml.) for 2 hr. The mixture was poured into water, the moist acetate was refluxed with ethanol (50 ml.), and the insoluble portion filtered off from the hot solution. The filtrate, on concentration, yielded an acetate, m. p. 180—190°. The alcohol-insoluble acetate (4·5 g.) crystallised from benzene–ethanol and then from ethyl acetate as plates, m. p. 248—250° (Found: C, 82·0; H, 11·3. Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2%), $[\alpha]_D^{18}$ +89·2° (*c* 1·178). When this acetate (1·8 g.) was refluxed in benzene (30 ml.) and 5% ethanolic potassium hydroxide (30 ml.) for 2 hr., taraxasterol, m. p. 222—225° (from ethanol), was recovered (Found: C, 84·4; H, 11·7. Calc. for $C_{30}H_{50}O$: C, 84·4; H, 11·8%), $[\alpha]_D^{20}$ +93·5° (*c* 1·33). Taraxasteryl benzoate was obtained when the sterol (0·5 g.) was heated with pyridine (5 ml.) and benzoyl chloride (1 ml.) for 3 hr. It formed needles, m. p. 252° (from benzene–ethanol; ethyl acetate) (Found: C, 83·6; H, 10·3. Calc. for $C_{37}H_{54}O_2$: C, 83·7; H, 10·25%), $[\alpha]_D^{20}$ +103·5° (*c* 1·206). Hydrolysis with ethanolic potassium hydroxide led to taraxasterol, m. p. 222—225°. The *p*-nitrobenzoate, prepared by heating a solution of taraxasterol (0·4 g.) in pyridine (10 ml.) with *p*-nitrobenzoyl chloride (1·5 g.) for 4 hr., formed whitish plates, m. p. 277—278° (from benzene–ethanol; ethyl acetate) (Found: C, 77·4; H, 9·1. Calc. for

[13] Wall, Eddy, McClenna, and Klumpp, *Analyt. Chem.*, 1952, **24**, 1337; Wall, Eddy, and Klumpp, *ibid.*, 1953, **25**, 266.

[14] Jones, Katenzellenbogen, and Dobriner, *J. Amer. Chem. Soc.*, 1953, **75**, 158.

$C_{37}H_{53}NO_4$: C, 77·2; H, 9·2%), $[\alpha]_D^{18}$ +103° (*c* 1·29). Hydrolysis of this ester gave pure taraxasterol.

Preparation of ψ-Taraxasterol.—The acetate (2 g.), recovered from the first benzene–ethanol mother liquor after separation of taraxasterol acetate, was hydrolysed with ethanolic potassium hydroxide–benzene; the crude sterol (1·2 g.) was benzoylated as before, the benzoate boiled with methanol (30 ml.), and the insoluble portion separated. This formed needles, m. p. 270°, from benzene–ethanol and ethyl acetate (Found: C, 83·9; H, 10·1. Calc. for $C_{37}H_{54}O_2$: C, 83·7; H, 10·25%), $[\alpha]_D^{19}$ +74·6° (*c* 1·355). Hydrolysis of this benzoate yielded the free sterol, needles, m. p. 210°, from ethanol.

ψ-Taraxasterol Formate.—The foregoing sterol (1 g.) was refluxed with benzene (10 ml.) and anhydrous formic acid (10 ml.) for 2 hr. After dilution with water and ether, the ethereal solution was separated and washed with sodium hydrogen carbonate. ψ-Taraxasteryl formate crystallised from acetone and from benzene–ethanol as needles, m. p. 219—222° (Found: C, 82·3; H, 11·1. Calc. for $C_{31}H_{50}O_2$: C, 81·9; H, 11·1%), $[\alpha]_D^{21}$ +41·4° (*c* 1·556). ψ-Taraxasterol was prepared by hydrolysis of the formate (0·5 g.) with benzene–ethanolic potassium hydroxide and crystallised from ethanol in needles, m. p. 218—219° (Found: C, 84·4; H, 11·5. Calc. for $C_{30}H_{50}O$: C, 84·4; H, 11·8%), $[\alpha]_D^{23}$ +41·6° (*c* 1·135). Its benzoate crystallised from ethyl acetate as needles, m. p. 277° (Found: C, 84·1; H, 10·1. Calc. for $C_{37}H_{54}O_2$: C, 83·7; H, 10·25%), $[\alpha]_D^{23}$ +67·2° (*c* 1·27). ψ-Taraxasteryl *p*-nitrobenzoate crystallised from benzene–ethanol followed by ethyl acetate as plates, m. p. 275°.

Conversion of Taraxasterol into ψ-Taraxasterol.—When taraxasterol (0·9 g.) was refluxed with benzene (10 ml.) and formic acid (10 ml.) for 2 hr. and the mixture worked up as before, ψ-taraxasteryl formate, m. p. and mixed m. p. 219—221°, was obtained. Hydrolysis of this formate led to ψ-taraxasterol, m. p. and mixed m. p. 217—218°.

Chromatography of the Brown Resin.—A solution of the resin (20 g.) in benzene (200 ml.) was filtered through a column (2·5 × 45 cm.) of alumina (180 g.) (Riedel-de Haen, A.G.; " Standardised for Chromatographic Adsorption "), heated for 2 hr. at 120° before being used. Benzene (2 l.) was then percolated through, and the process was carried out on two identical columns, equivalent fractions (each from 200 ml. of benzene) being combined.

The first four fractions were treated with acetone leaving a white crystalline polyterpene, m. p. ~160°.

The remaining fractions were dissolved in acetone, the solution heated with animal charcoal, and the filtrate concentrated, giving a phytosterol (2 g.) as plates, m. p. 133—135°.

Each of the two columns was divided into two sections (20 and 25 cm.) which were extracted with boiling ethanol. Dark brown material (3·3 g.) was recovered from the top section, whilst the second yielded a solid (4·2 g.) from which the above-mentioned phytosterol (1 g.), m. p. 133—135°, was obtained on crystallisation from methanol.

Fractionation of the Phytosterol.—(a) *As benzoate.* A solution of the phytosterol (2 g.) in pyridine (20 ml.) was heated with benzoyl chloride (6 ml.) for 4 hr., and the benzoate was digested with methanol. On fractional crystallisation of this benzoate (m. p. 136°) from benzene—methanol, stigmasteryl benzoate was obtained as plates, m. p. 164—165° (lit.,[15] m. p. 156—158°) (Found: C, 83·5; H, 10·15. Calc. for $C_{36}H_{52}O_2$: C, 83·7; H, 10·1%), $[\alpha]_D^{30}$ −28·8° (*c* 0·556). Stigmasterol was obtained by hydrolysis of this benzoate with benzene–ethanolic potassium hydroxide; it crystallised from ethanol as plates, m. p. and mixed m. p. 168—170° (Found: C, 84·7; H, 11·7. Calc. for $C_{29}H_{48}O$: C, 84·4; H, 11·8%). The acetate crystallised from benzene–ethanol in plates, m. p. and mixed m. p. 140—141°[16] (Found: C, 82·1; H, 10·85. Calc. for $C_{31}H_{50}O_2$: C, 81·85; H, 11·1%).

(b) *As 3,5-dinitrobenzoate.* A solution of the phytosterol (1 g.) in pyridine (10 ml.) was heated with 3,5-dinitrobenzoyl chloride (3 g.) for 4 hr. and the ester was digested with hot ethanol. After several crystallisations from chloroform–ethanol, benzene–ethanol, and ethyl acetate, stigmasteryl 3,5-dinitrobenzoate was obtained as yellowish-white plates, m. p. and mixed m. p. 228°. A specimen prepared from the pure stigmasterol of Soya beans[16] had m. p. 230° (lit.,[6] m. p. 234—235°, Kofler block) (Found: C, 71·0; H, 8·1; N, 4·8. Calc. for $C_{36}H_{50}N_2O_6$: C, 71·3; H, 8·3; N, 4·6%). Hydrolysis yielded stigmasterol, m. p. and mixed m. p. 168—170°.

β-*Sitosterol.*—β-Sitosteryl 3,5-dinitrobenzoate was recovered from the first two mother liquors left after separation of stigmasteryl 3,5-dinitrobenzoate; it crystallised from ethyl

[15] Asselineau and Asselineau, *Bull. Soc. chim. France*, 1957, 11, 12, 1359.
[16] Zaki and Soliman, *J.*, 1940, 1545.

acetate as yellowish-white plates, m. p. and mixed m. p. 205° (Found: C, 70·7; H, 8·3; N, 4·65. Calc. for $C_{36}H_{52}N_2O_6$: C, 71·0; H, 8·6; N, 4·6%). Hydrolysis of this ester gave β-sitosterol, in plates, m. p. 137° (from ethanol) (Found: C, 84·2; H, 12·0. Calc. for $C_{29}H_{50}O$: C, 84·0; H, 12·2%), and its benzoate, plates, m. p. 148° (from benzene–ethanol) (Found: C, 83·3; H, 10·4. Calc. for $C_{36}H_{54}O_2$: C, 83·3; H, 10·5%).

Extraction of the Leaves with Light Petroleum.—The crushed air-dried leaves (10 kg.) were extracted (Soxhlet) with light petroleum, and the green extract distilled. The residue (240 g.) was mixed with ethanol (1 l.) and the mixture heated to boiling, cooled, and filtered. The waxy residue was again treated with ethanol (1 l.) and the combined filtrates concentrated to about 600 ml. The concentrate was hydrolysed with potassium hydroxide (80 g.) for 2 hr. and, after concentration, the nonsaponifiable material was extracted with ether and recovered as a yellowish-brown residue (45 g.). A solution of this residue (14 g.) in light petroleum (400 ml.) was filtered through two identical columns of alumina as previously described. Each column was developed with light petroleum (1 l.) and then eluted with benzene (2 l.). The light petroleum yielded wax (0·5 g.) whereas equivalent benzene fractions, collected in 200-ml. portions from each column, were combined.

Taraxasterol, m. p. 217—220°, was isolated from fractions 7—10 by crystallisation from benzene and then from ethanol, and with greater difficulty from fractions 3—6. It was characterised as the acetate and the benzoate, as before.

The Phytosterol Mixture.—Each column, after elution with benzene, was divided (from the top) into three sections (9, 18, and 18 cm. in length) which were extracted with boiling ethanol. The first section yielded a resin (1·7 g.) whereas the second (2·4 g.) and third (2·2 g.) afforded the phytosterol (1·8 g.) on treatment with ethanol or acetone. This crystallised from ethanol as plates, m. p. 136°, which yielded stigmasterol and β-sitosterol when fractionated as benzoate or 3,5-dinitrobenzoate.

Wax A. The alcohol-insoluble waxy residue (100 g.) was washed with ice-cold ether, the insoluble greenish-white residue (50 g.) was dissolved in benzene and heated with animal charcoal, and the hot filtrate diluted with acetone. On cooling, the wax separated and recrystallised from benzene–acetone as clusters, m. p. 68° [Found: C, 81·5; H, 13·6%; *M* (Rast), 740. $C_{44}H_{88}O_2$ requires: C, 81·4; H, 13·7%; *M*, 649. $C_{46}H_{92}O_2$ requires C, 81·6; H, 13·7%; *M*, 677]. It was recovered after being heated with acetic anhydride.

Wax B. The greenish semi-solid (50 g.) recovered from the ethereal washings of the foregoing wax was refluxed with 10% ethanolic potassium hydroxide (400 ml.) for 2 hr., and the extract concentrated to about 200 ml., diluted with water, and extracted with ether. The nonsaponifiable material (25 g.), recovered from the ethereal solution, was dissolved in hot ethanol, boiled with animal charcoal, and filtered. The wax which separated on cooling recrystallised from benzene–acetone as needles, m. p. 68° [Found: C, 85·05, 85·2; H, 14·7, 14·6%; *M* (Rast) 502, 514. $C_{32}H_{66}$ requires C, 85·2; H, 14·8%; *M*, 450·5. $C_{34}H_{70}$ requires C, 85·3; H, 14·7%; *M*, 478·5].

Extraction of the Receptacles with Alcohol.—The defatted receptacles (2 kg.) were extracted with boiling ethanol, and the extract, which deposited sodium chloride on cooling, was concentrated to a thick syrup. This was warmed with water (250 ml.); the residue was dried, defatted with light petroleum, and then mixed with a saturated solution of sodium hydrogen carbonate (200 ml.). When the mixture ceased to effervesce, the brown residue was washed and dried. On acidification of the bicarbonate solution, a reddish-brown resin separated. The alcoholic extract of 10 kg. of the defatted receptacles thus yielded 50 g. of residual fatty material, 14 g. of neutral resin, and 50 g. of acidic material.

The aqueous solution gave a green colour with ferric chloride, and an osazone, m. p. 218°, which was converted into glucose osotriazole, m. p. and mixed m. p. 197°.

Isolation of Cynarogenin.—A solution of the neutral resin (14 g.) in benzene–ethanol (200 ml. of each) was refluxed with animal charcoal, and the filtrate concentrated until a brown amorphous precipitate separated. This process was repeated until the sapogenol was obtained as whitish microcrystals (2 g.), m. p. 295—300° (decomp.).

Cynarogenin Acetate.—This was obtained when the sapogenol (0·8 g.) was heated in pyridine (10 ml.) and acetic anhydride (10 ml.). It crystallised from light petroleum or methanol as glistening plates, m. p. 163° [Found: C, 69·0, 68·9; H, 9·0, 9·1; Ac, 22·3, 22·6%; *M* (Rast), 587. $C_{33}H_{50}O_8$ requires C, 69·0; H, 8·8; 3Ac, 22·5%; *M*, 574]. Pure cynarogenin was prepared by hydrolysis of this acetate with benzene–ethanolic potassium hydroxide. It crystallised from

benzene–ethanol as plates, m. p. 305° (decomp.), identical with the original sapogenol (Found: C, 72·6, 72·7; H, 10·15, 10·0. $C_{27}H_{44}O_5$ requires C, 72·3; H, 9·9%). This sapogenol was partly dehydrated on being heated in a high vacuum over phosphorus pentoxide at 120° (Found: C, 73·4; H, 10·0%).

Cynarogenin benzoate was prepared when a solution of the sapogenol (0·5 g.) in pyridine (10 ml.) was heated with benzoyl chloride (1·5 ml.) for 4 hr. It was recovered as usual and refluxed with methanol, and the insoluble fraction crystallised from benzene–ethanol as plates, m. p. 195° (Found: C, 76·1, 76·0; H, 7·7, 7·5. $C_{48}H_{56}O_8$ requires C, 75·8; H, 7·4%), $[\alpha]_D^{21}$ −17·0° (*c* 1·41). Hydrolysis yielded cynarogenin, m. p. 305° (decomp.).

The *p*-nitrobenzoate was obtained when a solution of the sapogenol (0·5 g.) in pyridine (10 ml.) was heated with *p*-nitrobenzoyl chloride (1·5 g.) for 4 hr. on the water-bath. The mixture was then poured into water, the solid washed with sodium hydrogen carbonate solution and with water, and then boiled with methanol and the ester filtered off hot. It crystallised from benzene–ethanol as needles, m. p. 267° (Found: C, 64·1, 64·3; H, 6·2, 6·2; N, 4·7, 4·6. $C_{48}H_{53}N_3O_{14}$ requires C, 64·4; H, 6·0; N, 4·7%).

Extraction of the Leaves with Alcohol.—The defatted leaves (5 kg.) were extracted with boiling ethanol and the extract was concentrated to a dark green viscous residue; sodium chloride separated from this extract. This residue was heated on the water-bath with water (1·7 l.). The water-insoluble residue was freed from residual fat and separated into a neutral resin (11·5 g.) and a dark greenish acidic material as for the receptacles. The former yielded cynarogenin, m. p. 305° (decomp.), characterised as acetate and benzoate.

Isolation of a Cynarine-like Substance.—The orange aqueous solution, which gave a green colour with ferric chloride, was mixed with 20% lead acetate solution (450 ml.). The moist yellow lead complex was digested with 40% acetic acid (450 ml.) and the solution saturated with hydrogen sulphide. On concentration of the filtrate to about 30 ml. under reduced pressure, a yellowish-brown precipitate separated on cooling. When the dry precipitate was extracted with methanol and the solution concentrated, a yellow substance separated out; cynarine was recovered on further concentration; it crystallised from methanol as needles, m. p. 227°. The yellow compound crystallised from methanol as yellowish needles, m. p. 250°, which gave a green colour with ferric chloride and decomposed sodium hydrogen carbonate (Found: C, 51·9, 52; H, 5·3, 5·4%). Its acetate crystallised from ethyl acetate as fine needles, m. p. 240° [Found: C, 56·55; H, 4·8; Ac, 45·6%; *M* (Rast), 418, 430], $[\alpha]_D^{16}$ −32·1° (*c* 1·52).

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