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Herbal Remedies of the Maritime Indians: Sterols and Triterpenes of *Achillea millefolium* L. (Yarrow)

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Received July 23, 1981, from the **College of Pharmacy and the ‡Department of Chemistry, Dalhousie University, Halifax, Nova Scotia, B3H 3J5*, and the *§Atlantic Research Laboratory, National Research Council, Halifax, Nova Scotia, B3H 3Z1.* Accepted for publication October 1, 1981. ¶ Present address: Department of Chemistry, University of Guelph, Guelph, Ontario, Canada N1G 2W1.

Abstract ☐ As part of ongoing studies of the medicinal aspects of Maritime flora, particularly the herbal remedies of the Micmac and Malecite Indians, the sterols and triterpenes of *Achillea millefolium* L. (Compositae), a widely used herbal remedy known commonly as yarrow, were determined. Using modern techniques, including nuclear magnetic resonance spectroscopy and combined GC–mass spectrometry, β-sitosterol was identified as the major sterol and α-amyrin as the major triterpene of this plant. The sterols stigmasterol, campesterol, and cholesterol and the triterpenes β-amyrin, taraxasterol, and pseudotaraxasterol were also identified. Successful therapeutic application of yarrow may be partly due to the presence of one or more of these compounds since many sterols and triterpenes exhibit a wide range of pharmacological activities. This is the first reported occurrence of cholesterol, campesterol, and the four triterpenes in yarrow.

Keyphrases ☐ *Achillea millefolium* L.—herbal remedies, extraction of triterpenes and sterols ☐ Triterpenes—extraction from *Achillea millefolium* L., herbal remedies, sterols ☐ Sterols—extraction from *Achillea millefolium* L., herbal remedies, triterpenes

Since the Trojan war (~1200 BC), *Achillea millefolium* L. has been used extensively by many cultures on different continents as a herbal remedy for various afflictions (1). While conducting studies on the medicinal aspects of the Maritime flora, particularly the herbal remedies of the Micmac and Malecite Indians (2, 3), it was observed that although *A. millefolium* (yarrow) had been widely used and extensively studied (1), very little had been reported regarding its sterols and triterpenes.

Some early articles and various screening papers have reported sterols, triterpenes (3–5), and saponins (6, 7) present in yarrow, although at least one paper stated that the plant contained no steroids (8). Only two authors have previously attempted to determine the nature of the sterols and triterpenes present in this plant. One of those authors documented the presence of stigmasterol and a sitosterol (9), while another reported the presence of β-sitosterol, its acetate, a phytol, and a diol (10).

Therefore, the examination of yarrow for the presence and nature of these compounds is described.

EXPERIMENTAL

Collection and Extraction—The aerial parts of *A. millefolium* were collected during the flowering stage from an open field near Scots Bay, Kings Co., Nova Scotia in September, 1979[1]. The plant material was dried in a forced-air oven at 60° and ground in a Wiley mill to a coarse powder (0.5 cm). This material (2.09 kg) was placed in a stainless steel tank and

[1] The plant material utilized in this investigation was identified as *Achillea millefolium* (L.) (Compositae) by Dr. M. J. Harvey, Department of Biology, Dalhousie University. Herbarium samples (Number 79-03) representing material collected for this investigation are available for inspection at the College of Pharmacy, Dalhousie University.

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Table I—Nuclear Magnetic Resonance Assignments for Yarrow Triterpene Acetates

Group Assignment	α-Amyrin		β-Amyrin		Taraxasterol		ψ-Taraxasterol	
	Identity[a]	Shift[b]	Identity[a]	Shift[b]	Identity[a]	Shift[b]	Identity[a]	Shift[b]
CH_3—C	3H,s	0.81	6H,s	0.84	9H,s	0.85	3H,s	0.74
CH_3—C	9H,s	0.89	12H,s	0.89	3H,s	0.87	3H,s	0.85
CH_3—C	3H,s	0.93	—	—	3H,s	0.93	3H,s	0.86
CH_3—C	3H,s	1.00	6H,s	0.98	—	—	3H,s	0.88
CH_3—C	3H,s	1.02	—	—	3H,s	1.02	3H,s	0.95
CH_3—C	3H,s	1.08	—	—	—	—	3H,s	1.05
CH_3—C	—	—	—	—	3H,d[c]	1.02	3H,d[c]	1.04
CH_3—C=	—	—	—	—	—	—	3H,s	1.66
CH_2—CH=C	2H,m	1.90	2H,m	1.90	—	—	—	—
CH_3—CO	3H,s	2.06	3H,s	2.06	3H,s	2.05	3H,s	2.05
CH_3—CH	—	—	—	—	1H,m	2.10	1H,m	2.10
CH_2=C—CH	—	—	—	—	1H,m	2.40	—	—
CH—OAcetate	1H,t[d]	4.53	1H,t[d]	4.53	1H,t[d]	4.50	1H,q[e]	4.50
CH_2=C	—	—	—	—	2H,m	4.60	—	—
CH=C	1H,t[f]	5.14	1H,t[g]	5.18	—	—	1H,d[c]	5.27

[a] In deuterochloroform; number of hydrogens, multiplicity (d = doublet, m = multiplet, q = quartet, s = singlet, t = triplet), J = coupling constant. [b] δ, ppm. [c] J = 6 Hz. [e] J = 7, 11 Hz. [f] J = 3–4 Hz. [g] J = 6 Hz.

macerated in chloroform–methanol (1:1, v/v) for at least 24 hr; the extract was then drained from the tank. This process was repeated 3 times; the extracts were then combined and the solvent removed *in vacuo*, producing a dark green, waxy mass (78.8 g). This material was saponified following a published method (11). The nonsaponifiable portions were extracted with ether and the solvent was removed *in vacuo*, yielding an amber-colored residue (34.2 g).

Thin-Layer Chromatography—The nonsaponifiable portions were fractionated by preparative TLC using plates coated with silica gel[2] (11). Following visualization procedures, the sterols appeared as a red band ($R_f \simeq 0.25$) and the triterpenes as a red-brown band $R_f \simeq 0.40$). The material from these two bands was recovered by extracting the silica gel with ether and chloroform (12).

The triterpene band, converted to acetates (12), was further fractionated using silica gel[2] impregnated with silver nitrate (13). The visualization process, using 2′,7′-dichlorofluorescein (13), indicated that six bands were present; these were numbered according to increasing R_f. Following recovery from the silica gel with ether and chloroform, bands 3, 4, and 5 gave positive Liebermann-Burchard tests (14) for triterpenes. Bands 1, 2, and 6 produced negative tests and were not considered for further study.

Gas–Liquid Chromatography—The sterol fraction was analyzed using SE-30, OV-1, OV-17, and OV-225[3] columns[4], authentic reference standards, and cholestane as an internal standard (15). The triterpenes were similarly analyzed using SE-30, OV-17, and OV-225 columns and respective oven temperatures of 290, 275, and 250°. The acetates (12) of the taraxasterols and their dihydroderivatives were analyzed using OV-1 with an oven temperature of 275° and OV-17 and OV-225 columns.

Nuclear Magnetic Resonance Spectroscopy—All spectra were recorded in dilute solutions in deuterated solvents using tetramethylsilane as the internal reference[5,6].

Gas Chromatography–Mass Spectrometry—GC–mass spectrometry studies[7] were performed on a high-capacity 3% OV-1 flexible quartz capillary column[8] (25 m × 0.3-mm i.d.), which was directly interfaced to the source of the mass spectrometer. Electron impact mass spectra were determined at 70 eV and a source temperature of 260° (indicated). Source temperature was 230° (indicated) for the positive ion chemical ionization mass spectrometric studies. Methane was used as the ionizing gas at a source pressure of 0.02 torr (indicated), and helium was used as the carrier gas (2 ml/min).

Taraxasterol and pseudotaraxasterol acetates were also run on an OV-1 packed column[9] with an oven temperature of 290° and with helium as the carrier gas (35 ml/min).

Hydrogenation—Sufficient samples (2–3 mg) of taraxasterol acetate, pseudotaraxasterol acetate, and α-amyrin acetate fractions were dissolved in ethanol. Each was hydrogenated in a microhydrogenator[10] at room temperature and pressure using palladium–charcoal as the catalyst. After 3 hr, the reaction medium was filtered to remove the catalyst and reduced to dryness *in vacuo*.

Authentic Samples—Authentic samples of cholestane[11], β-sitosterol[11], stigmasterol[11], cholesterol[12], campesterol[13], α- and β-amyrin[14] were purchased; taraxasterol[15] and pseudotaraxasterol acetate[16] were gifts.

RESULTS AND DISCUSSION

Quantitative TLC indicated that sterols and triterpenes represented ~6 and 54%, respectively, of the nonsaponifiable materials in yarrow (11). GLC studies of the TLC fractions demonstrated that β-sitosterol was the major sterol present, representing ~75% of the sterols. Campesterol (10%), cholesterol (5%), stigmasterol (3%), and an unidentified sterol (6%) were also present. Sequential coinjection of the sterol fraction recovered from TLC with each of the authentic sterols produced single, symmetrical peaks, verifying the identity of these four sterols from yarrow.

Similar studies of the triterpene fraction using the SE-30 column indicated that there were four triterpenes in the material recovered from the TLC fractions. The OV-17 column presented a split peak, indicating the presence of at least five triterpenes. Complete separation, achieved on an OV-225 column, established that yarrow contained seven triterpenes. Comparison with authentic material verified that α-amyrin was the major triterpene present, representing ~42% of this fraction; β-amyrin (33%) and taraxasterol (18%) were also identified in this way. A fourth triterpene, tentatively identified as pseudotaraxasterol (4%), and three unidentified triterpenes accounted for the balance of this fraction (3%).

The triterpene fraction was acetylated (12) and subjected to argentation chromatography (13, 16, 17) to obtain sufficient amounts of pseudotaraxasterol to conduct instrumental and chemical analyses. Initially, six bands were obtained, although only bands 3, 4, and 5 produced positive Liebermann-Burchard tests (14) following elution from the silica gel. Bands 3, 4, and 5 were each subjected to further argentation chromatography. In this manner it was possible to obtain a few milligrams of α- and β-amyrin acetates from band 5, pseudotaraxasterol acetate from band 4, and taraxasterol acetate from band 3. Each of these samples was at least 80% pure, and nuclear magnetic resonance (NMR) (Table I) and GC–mass spectrometry (Tables II and III) data were collected for each.

The NMR spectra of α- and β-amyrin acetates and taraxasterol acetate in deuterochloroform and deuterobenzene were in keeping with similar compounds (18, 19).

The electron impact mass spectra of α- and β-amyrin acetate agreed

[2] Silica gel H, Brinkmann Instruments (Canada) Ltd., Toronto, Ontario, Canada, catalog number 7736.
[3] Three percent on 100–120 mesh Chromosorb W-HP.
[4] Chromatographic Specialties, Brockville, Ontario, Canada.
[5] Preliminary NMR spectra were recorded on Varian A-60, T-60, and CFT-20 spectrometers of Dalhousie University, Halifax.
[6] The 220 MHz NMR spectra were recorded on a Varian HR-220 spectrometer located at the Canadian 220 NMR Centre, Department of Medical Genetics, University of Toronto.
[7] Finnigan-MAT 4000 GC–MS quadrapole mass spectrometer coupled to an INCOS data system.
[8] Hewlett-Packard (Canada) Ltd., Mississauga, Ontario, Canada.
[9] Three percent on 100–120 mesh Gas Chrom. Q, 200 cm × 2 mm, Chromatographic Specialties, Brockville, Ontario, Canada.
[10] Supelco, Inc., Bellefonte, PA 16823.
[11] Sigma Chemical Co., St. Louis, Mo.
[12] Fisher Scientific Co., Montreal, Quebec, Canada.
[13] Applied Science, State College, Pa.
[14] Pfaltz and Bauer, Inc., Stamford, Conn.
[15] Professor T. R. Watson, Pharmacy Department, University of Sydney, Sydney, Australia.
[16] Dr. R. V. Madrigal, Northern Regional Research Laboratory, USDA, Peoria, Ill.

Table II—Relative Intensities in the Electron Impact Mass Spectra of Yarrow Triterpene Acetates

	Capillary Column					Packed Column	
m/z	α-Amyrin	β-Amyrin	Taraxasterol	Pseudo-taraxasterol	Dihydrotaraxasterol	Taraxasterol	Pseudo-taraxasterol
470[a]	—	—	—	—	0.3	—	—
468[a]	0.4	0.4	1.7	0.2	—	9.5	3.6
410	—	—	—	—	0.8	—	—
408	—	—	1.6	0.3	—	8.2	8.6
395	—	—	—	—	0.9	—	—
393	—	—	0.8	0.5	—	3.4	2.8
326	—	—	—	—	—	—	0.9
249	0.7	0.6	6.7	4.3	8.7	12	8.8
229	—	—	3.6	—	—	—	—
218	100	100	5.0	2.2	1.6	7.7	4.3
204	—	—	14	—	—	—	—
203	27	51	15	9.3	6.9	16	15
191	7.5	3.4	31	16	36	24	60
189	44	19	100	100	100	100	100
175	12	11	23	19	12	21	26
161	18	8.6	23	14	9.4	20	26
149	15	7.2	18	12	15	17	25
147	23	15	31	18	10	—	32
137	10	—	11	8.1	24	11	13
135	42	18	55	44	35	43	47
133	32	15	28	32	12	21	42
123	30	9.2	32	36	48	25	29
121	38	19	74	77	47	52	69
109	44	20	81	64	51	57	51
107	45	23	72	65	45	43	50

[a] Molecular ion (M+).

Table III—Relative Intensities in the Chemical Ionization Mass Spectra of Yarrow Triterpene Acetates [a]

m/z	α-Amyrin	β-Amyrin	Taraxasterol	Pseudotaraxasterol
468[b]	0.2	0.1	0.2	0.1
453	0.1	0.2	0.1	0.1
409	63	38	58	55
408	49	31	28	32
394	—	—	—	6.3
393	24	16	25	25
327	—	—	—	0.4
326	—	—	—	0.2
249	2.3	2.3	3.1	2.3
219	38	34	39	—
218	58	43	9.0	—
205	60	64	73	—
203	38	49	49	—
191	62	69	99	—
189	37	42	47	—
175	20	25	20	16
161	20	25	16	16
149	47	47	47	44
147	21	22	14	13
137	46	51	35	34
135	41	39	30	33
133	23	24	15	19
123	27	66	65	48
121	43	41	41	41
109	100	100	100	100
107	32	35	21	20

[a] Methane was the ionizing gas. [b] Molecular ion (M+).

with published data (13), while that of taraxasterol acetate was in keeping with predicted fragmentation patterns (20, 21). The positive chemical ionization mass spectra for these compounds differed only in the relative abundance of the fragment ions. All showed a base peak at m/z 109.

Direct comparison with authentic samples by GLC, NMR, and GC–mass spectrometry confirmed the identity of these three triterpene acetates.

The identification of pseudotaraxasterol (ψ-taraxasterol) was the most difficult and frustrating aspect of this research. Previous investigators also encountered this problem. As early as 1938 it was stated (22) that the material Power and Browning (23) had called homotaraxasterol was "a difficultly separable mixture of taraxasterol, ψ-taraxasterol, and possibly other unidentified substances."

The 220-MHz NMR spectrum provided valuable information that helped to identify this compound as pseudotaraxasterol. The spectrum of the acetate contained peaks for the acetate methyl group (2.05 ppm) and for the corresponding methine hydrogen (4.50 ppm) that were in keeping with the spectra described previously.

The spectrum also showed six methyl singlets present between 0.74 and 1.05 ppm. The three resonances that provided the most data, however, were those at 1.04, 1.66, and 5.27 ppm. The first was a three-proton doublet (J = 6 Hz), which corresponded to the features expected of a methyl group at a tertiary carbon. The signal at 1.66 ppm was not clearly defined, but contained a broad three-proton singlet corresponding to a vinylic methyl group. These groups normally appear in this region (1.63–1.80 ppm), and, although usually sharp and well defined (18), they may appear as a broad, less well defined peak at ~ 1.67 ppm (19). Irradiation experiments showed that a methine hydrogen coupled to the olefinic proton at 5.27 ppm also appeared at this resonance. This latter resonance was perhaps the most useful one in the entire spectrum. This one-proton signal appeared as a broad doublet (J = 6 Hz), the broadening presumably caused by additional coupling. The appearance of such a one-proton signal indicated that the other olefinic carbon was quaternary and either at a ring junction or bonded to a methyl group.

To determine the skeletal structure of the triterpene in question, a small amount was hydrogenated and the product was compared with those obtained by hydrogenating taraxasterol acetate and α-amyrin acetate. Of the limited number of compounds (Δ^5, Δ^9, Δ^{12}, Δ^{20}) that would produce the same product of catalytic hydrogenation as taraxasterol [$\Delta^{20(30)}$] and α-amyrin (Δ^{12}), only pseudotaraxasterol (Δ^{20}) fit the additional experimental facts presented by the NMR spectrum and by the mass spectrometry data to be discussed.

The GC–mass spectrometry data were obtained by both electron impact and methane positive chemical ionization. Although the diagnostic peak m/z 386 produced from cleavage of ring E (19) was absent in all spectra, a small peak at m/z 326 (386-acetic acid, 0.9%) occurred in the spectrum obtained by electron impact–mass spectrometry using a packed column and also at m/z 327 (0.4%) and 326 (0.2%) when positive chemical ionization–mass spectrometry and a capillary column were used. These peaks were not observed in the corresponding spectra of taraxasterol acetate. The hydrogenation experiment supplied further proof that the structure of pseudotaraxasterol was the correct assignment. Upon catalytic hydrogenation, both taraxasterol acetate and pseudotaraxasterol acetate produced a common product, dihydrotaraxasterol acetate. GC–mass spectrometry of these two products produced identical electron impact and positive chemical ionization spectra.

Conclusive proof of identity occurred when positive chemical ionization mass spectrometry and GLC on OV-17 and OV-225 columns showed that a sample of pseudotaraxasterol acetate[17] was identical to the pseudotaraxasterol acetate isolated from yarrow.

[17] A gift from Dr. R. V. Madrigal.

The second and smaller GLC peak obtained from the hydrogenation of taraxasterol acetate is believed to be the C-20 isomer (20α-methyl). This material produced a mass spectrum that was essentially identical to that of the other dihydrotaraxasterol acetate.

Thus, using modern instrumental techniques, four sterols and four triterpenes of yarrow were identified. β-Sitosterol was the major sterol and α-amyrin the major triterpene present. Other sterols identified were cholesterol, campesterol, and stigmasterol while the other triterpenes were β-amyrin, taraxasterol, and pseudotaraxasterol. Another study (24) found that pseudotaraxasterol occurred in *Tanacetum vulgare* L. (as did the other compounds) but it was not positively identified at that time due to sample size.

This study confirms that stigmasterol (9) and β-sitosterol (9, 10) are present in yarrow. It is also the first time that cholesterol, campesterol, α- and β-amyrin, taraxasterol, and pseudotaraxasterol have been reported in yarrow, although the presence of sterols and triterpenes have been previously noted (3–5, 10). No diols (10) were identified, although the possibility remains that they may have been the compounds observed with the longer relative retention time.

Much of the time and effort of this project was spent applying modern instrumental techniques to resolve a problem that has plagued chemists for over half a century—how to separate and identify taraxasterol and pseudotaraxasterol (22, 23).

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