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Lichen substances from subfossil and recent *Umbilicaria cylindrica*

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Abstract: Subfossil and recent thalli of the lichen *Umbilicaria cylindrica* from Greenland and other localities have been analysed for their secondary metabolites. Both appeared to belong to the same chemical strain.

Introduction

One of us (V. A.) recently detected at the west coast of Greenland (Fig. 1) subfossil thalli of the lichen *Umbilicaria cylindrica* (L.) Delise ex Duby which had been covered for about 1350 years by ice (ALSTRUP 1994). *U. cylindrica* had been growing at the locality when the ice cap was formed; it formed a major part of the subfossil vegetation and it had not been physically damaged by the ice. It was of interest to analyse chemically these thalli and to compare their metabolites with the secondary compounds from recent plants collected in Greenland, Canada, Russia, Scotland, Austria, and Switzerland. Here we report on our results.

Umbilicaria cylindrica was analysed several times: LINDBERG *et al.* (1953) found arabitol and mannitol in Swedish material while KROG (1968) could not detect any lichen substances by means of microcrystallization in plants from Alaska. RIPPERGER and HUNECK (1976) recorded ergosterol, lichesterol, and an unidentified sterol from a sample collected in the Tatra Mountains in the Slovak Republic. According to PURVIS *et al.* (1992) *U. cylindrica* from Great Britain contains norstictic acid sometimes.

Materials and methods

Subfossil material

North Greenland, Qaanaaq (Thule), 69°W, 77°30'N. The subfossil lichen (0.648 g) was pulverized and extracted with boiling acetone, yielding a weak yellowish oil (0.0004 g, 0.062%) which showed in the TLC (Merck SiO_2, benzene:dioxane:acetic acid = 18:5:0.8) two main spots of the R_F values 0.28 and 0.41 (red colouration with bis-diazotized benzidine and NaOH) and minor spots at the R_F values 0.51, 0.61, 0.69, and 0.89. The main spots could be identified by co-chromatography as gyrophoric and lecanoric acids. The oily extract was acetylated with acetic anhydride:pyridine = 1:1 (0.5 ml), the reagents were removed *i. vac.*, the residue was dissolved in methanol (3 ml), filtered through a Varian Bond Elut LRC DEA cartridge, the solvent removed *i. vac.* and the resulting mixture of sterol acetates analysed by GLC-MS.

Recent specimens

a) Greenland, NE Qaanaaq, *leg. et det. E. S. Hansen*, 1986. The sample (5.58 g) was pulverized and extracted with boiling diethyl ether for 36 hours; the separated crystals (A, 0.002 g, 0.003%) were removed by filtration and washed with Et_2O, acetylated with Ac_2O-pyridine and analysed by GLC. They proved to be a mixture of D-arabitol (75%) and D-mannitol (25%). The solvent of the filtrate was removed *i. vac.* and the resulting greenish oil (0.05 g, 0.89%) analysed by TLC (see under 1): nine spots of the R_F values 0.30, 0.45, 0.55, 0.64, 0.67, 0.70, 0.80, 0.89, and 0.98. The main component was gyrophoric acid. The oily residue was acetylated with Ac_2O-pyridine (like under 1) and analysed by GLC-MS.

b) Canada, North Baffin Island, on rocks along river bank of the Lewis River, 74°56'W, 70°24'N; *leg. et det. D. Fahselt*, 30.7.1991. The sample (3.17 g) was worked up like under a) and showed in the TLC 4 spots of the R_F values 0.39, 0.45, 0.54, and 0.66, neither of them identical with gyrophoric acid. The ethereal extract was acetylated with Ac_2O-pyridine and analysed by GLC-MS.

c) Russia, Murmansk Oblast, Kola Peninsula, Kirowsk, Chibiny Mountains, Yukspor Mt, *leg. et det. S. Huneck*, 29.6.1990. The sample (14.58 g) was worked up like under a): the extract (0.2 g, 1.37%) showed in the TLC 6 spots with the R_F values 0.37 (gyrophoric acid), 0.48 (lecanoric acid), 0.57, 0.61, 0.65 (orsellinic acid), and 0.88.

d) Scotland, Glencoe, *leg. S. Huneck*, 8.6.1991, *det. A. Fryday*, 1992; reactions: medulla and cortex NaOCl -, PD -. The Et_2O extract from 3 g lichen showed in the TLC the presence of a very small amount of orsellinic acid.

e) Austria, Steiermark, Handalpe, SW of Graz, *leg. S. Huneck*, 7.10.1990, *det. J. Poelt*, 1990. The Et_2O extract showed the presence of a small amount of orsellinic acid.

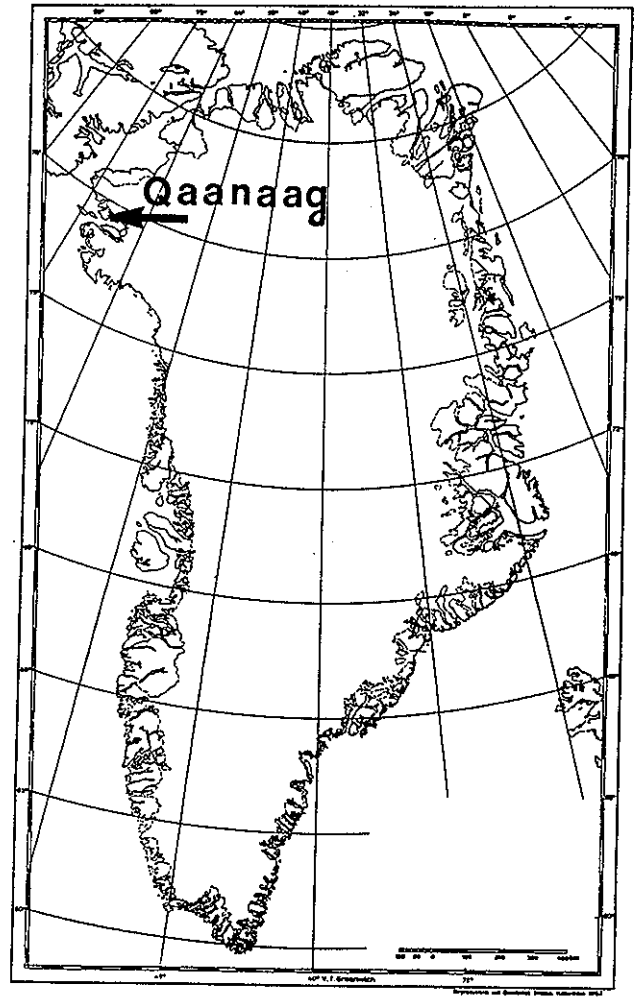


Fig. 1. Location of Qaanaaq (Thule) in Greenland.

f) Switzerland, Wallis, Champex, on rocks above the Alpine Botanical Garden, 1500 m, *leg. et det. S. Huneck*, 24.6.1993. The Et_2O extract from 26.53 g lichen showed in the TLC no phenolic compounds and was saponified by heating with methanolic KOH (10%, 5 ml, 2 hours). After usual work up the neutral fraction (0.04 g) was actylated with Ac_2O-pyridine and analysed by GLC-MS.

GLC of the acetates of D-arabitol and D-mannitol: Carlo Erba MEGA 5160; column PB-1 (Werner Günther Analysentechnik), 50 m x 0.32 mm, 0.25 µm film thickness, injection temperature 180 °C, column temperature 180 - 270 °C (isothermal), FID detection (290 °C), N_2, 1.5 ml/min., split injection (split ratio 1:20).

GLC-MS of the sterols and triterpenes: MD 800 (Fisons Instruments), EI (70 eV), source temperature 200 °C, column DB-1 (15 m x 0.25 mm, 0.25 µm film thickness), injection temperature 250 °C, interface temperature 300 °C, carrier gas He, flow rate 0.8 ml/min. Temperature program: 170 °C for 1 min., then elevated to 270 °C (25°/min.) and finally raised to 290 °C (2°/min.).

EI-MS: AMD 402 (AMD Intectra).

Results and discussion

Tab. 1 contains the phenolics and Tab. 2 the sterols and triterpenes found in subfossil and recent specimens of *Umbilicaria cylindrica*, including the relative retention times (RRT) of the sterols and triterpenes. In Tab. 3 the mass spectral data of sterols are summarized.

Tab. 1. Phenolics found in subfossil and recent specimens of *Umbilicaria cylindrica*.

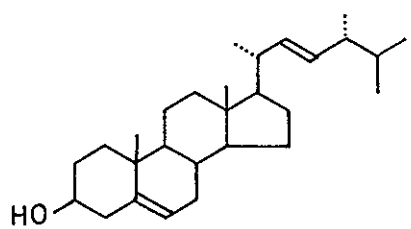
a_1: Greenland, subfossil
a_2: Greenland, recent
b: Canada
c: Russia
d: Scotland
e: Austria
f: Switzerland

Phenolic compound	a_1	a_2	b	c	d	e	f
Gyrophoric acid	++	++	-	++	-	-	-
Lecanoric acid	+	-	-	+	-	-	-
Orsellinic acid	-	+	-	+	(+)	(+)	-

Tab. 2. Sterols and triterpenes found in subfossil and recent specimens of *Umbilicaria cylindrica*.
F: Formula; RRT: Relative retention time; a_1: Greenland, subfossil; a_2: Greenland, recent; b: Canada; f: Switzerland

Sterols	F	RRT	a_1	a_2	b	f	
Brassicasterol	1	1.45	+	+	-	-	
Campesterol	2	1.54	+	+	+	+	
Cholesterol	3	1.39	+	+	+	+	
Ergosta-7,22-dien-3β-ol	4	1.52	+	-	-	+	
Ergosta-7,24(28)-dien-3β-ol	5	1.60	-	-	-	+	
Ergosta-5,7,9(11),22-tetraen-3β-ol (?)	6	1.44	+	+	+	+	AKIHIRA *et al.* 1992 DELSETH *et al.* 1979
Ergosta-4,6,8,11,22-pentaen-3β-ol*	7	1.47	+	+	+	+	
Ergosterol	8	1.50	-	-	-	+	
Lichesterol	9	1.46	-	-	-	+	
24-Methyl-23-dehydrolophenol	10	1.70	-	-	+	+	ITOH *et al.* 1981
4-Methylergosterol	11	1.70	-	-	+	-	
Sitosterol	12	1.69	+	+	+	+	
Stigmasterol	13	1.59	-	-	+	+	
Cholesta-3,5-dien-7-one	14	1.33	+	+	-	-	
Ergosta-3,5-dien-7-one	15	1.48	+	+	-	-	
Stigmasta-3,5-dien-7-one	16	1.62	+	+	-	-	ROWE 1965
Stigmasta-3,5,22-trien-7-one	17	1.53	+	+	-	-	
Triterpenes							
Zeorin	18	2.33	+	+	-	-	
3- or 6-Hydroxyhopane (?)		2.21	-	+	-	-	
3- or 6-Hydroxyhop-21(22)-ene		2.59	-	+	-	-	

* According to the mass spectrum 4 double bonds should be located in the rings A, B, and C.



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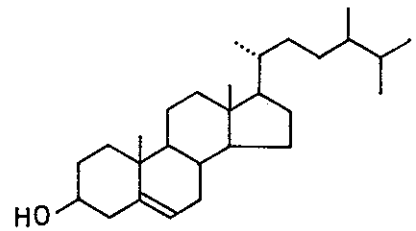

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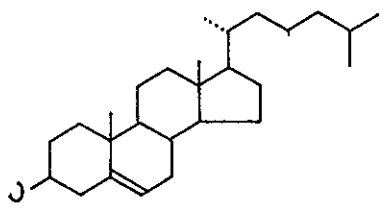

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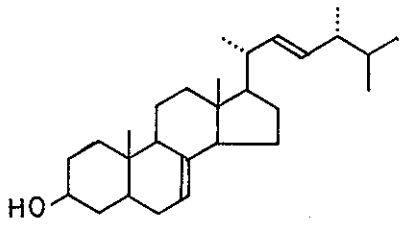

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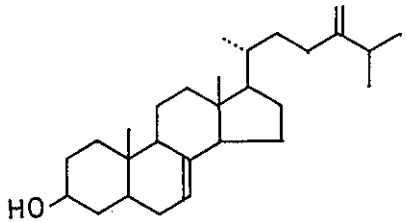

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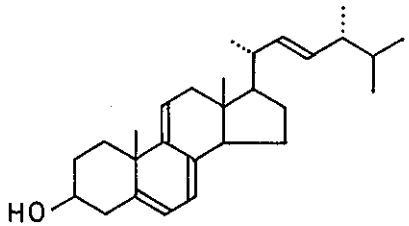

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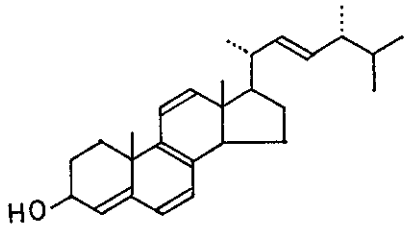

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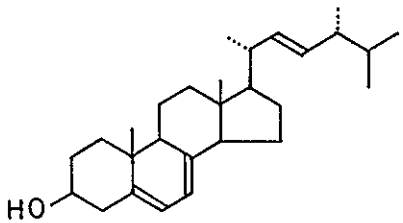

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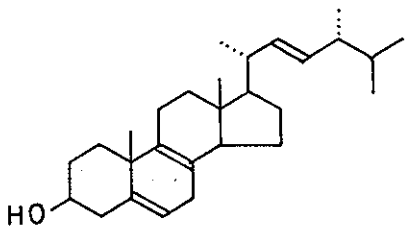

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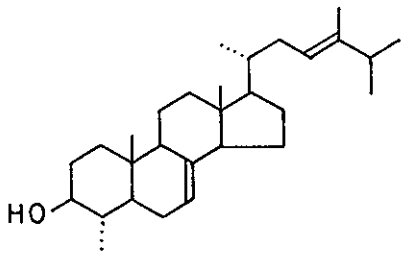

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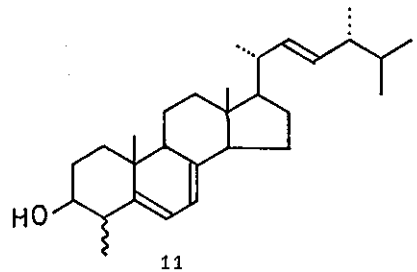

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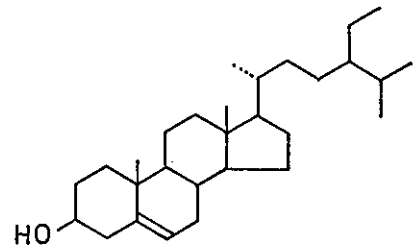

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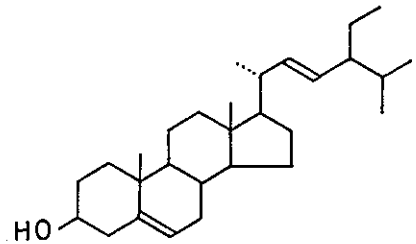

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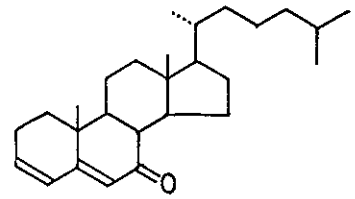

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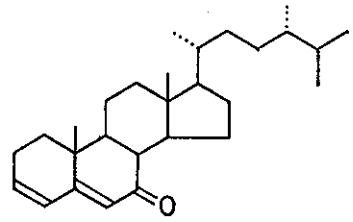

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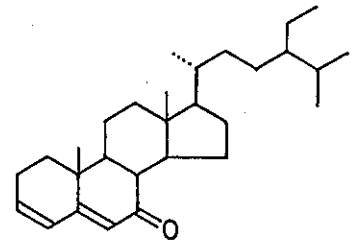

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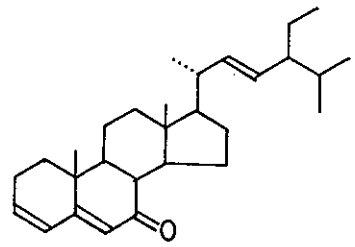

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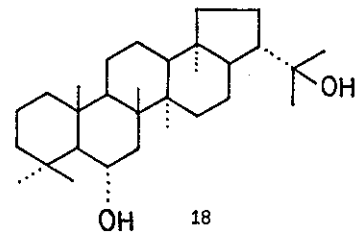

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Tab. 3. MS data of sterols from *Umbilicaria cylindrica*.

Cholesterol acetate. MS m/z (rel. int.): 368 ([M-HOAc]$^+$, 100), 353 (26), 260 (45), 255 (42), 247 (43), 213 (32), 159 (33), 147 (79).
Ergosta-5,7,9(11),22-tetraen-3β-ol acetate. MS m/z (rel. int.): 436 (M$^+$, 6), 376 ([M-HOAc]$^+$, 100), 361 (7), 291 (6), 251 (73), 235 (21), 209 (41), 195 (33), 179 (18), 165 (16), 153 (8), 69 (68).
Brassicasterol acetate. MS m/z (rel. int.): 380 ([M-HOAc]$^+$, 100), 365 (13), 337 (9), 282 (10), 255 (38), 228 (6), 213 (5), 145 (22), 105 (20).
Lichesterol acetate. MS m/z (rel. int.): 438 (M$^+$, 5), 378 ([M-HOAc]$^+$, 81), 363 (100), 337 (17), 253 (63), 211 (25), 199 (30), 157 (82), 147 (67), 143 (62).
Ergosta-4,6,8,11,22-pentaen-3β-ol acetate. MS m/z (rel. int.): 434 (M$^+$, 6), 374 ([M-HOAc]$^+$, 100), 359 (18), 261 (11), 249 (83), 233 (56), 219 (40), 205 (22).
Ergosterol acetate. MS m/z (rel. int.): 438 (M$^+$, 5), 378 ([M-HOAc]$^+$, 84), 363 (23), 337 (4), 253 (43), 211 (20), 157 (68), 143 (63), 109 (44), 81 (53), 69 (93), 55 (100).
Ergosta-7,22-dien-3β-ol acetate. MS m/z (rel. int.): 440 (M$^+$, 24), 425 (7), 380 ([M-HOAc]$^+$, 10), 342 (16), 313 (78), 288 (19), 255 (56), 229 (27), 213 (23), 161 (32), 105 (63), 81 (58), 69 (75), 55 (100).
Campesterol acetate. MS m/z (rel. int.): 382 ([M-HOAc]$^+$, 89), 367 (30), 274 (35), 271 (48), 261 (29), 255 (52), 213 (42), 147 (100).
4ξ-Methylergosterol acetate. MS m/z (rel. int.): 452 (M$^+$, 4), 392 ([M-HOAc]$^+$, 100), 311 (29), 267 (13), 239 (15), 197 (18), 69 (91), 55 (95).
Stigmasterol acetate. MS m/z (rel. int.): 394 ([M-HOAc]$^+$, 100), 379 (11), 351 (10), 282 (12), 255 (23), 253 (11), 228 (7), 211 (9), 159 (19), 83 (22).
Ergosta-7,24(28)-dien-3β-ol acetate. MS m/z (rel. int.): 440 (M$^+$, 5), 425 (11), 380 ([M-HOAc]$^+$, 3), 356 (20), 313 (100), 255 (17), 213 (26), 145 (22), 105 (53), 69 (67), 55 (82).
Sitosterol acetate. MS m/z (rel. int.): 396 ([M-HOAc]$^+$, 97), 381 (27), 354 (7), 313 (17), 288 (33), 275 (26), 255 (40), 213 (32), 147 (100).
24-Methyl-23-dehydrolophenol acetate. MS m/z (rel. int.): 454 (M$^+$, 5), 439 (13), 394 ([M-HOAc]$^+$, 3), 379 (12), 327 (100), 269 (17), 241 (19), 227 (31), 159 (22), 119 (42), 95 (63), 69 (61), 55 (88).
Cholesta-3,5-dien-7-one. MS m/z (rel. int.): 382 (M$^+$, 41), 367 (9), 269 (14), 227 (5), 187 (26), 174 (100), 161 (30), 134 (12).
Ergosta-3,5-dien-7-one. MS m/z (rel. int.): 396 (M$^+$, 50), 374 (11), 269 (18), 249 (16), 235 (6), 187 (28), 174 (100), 161 (35), 134 (11).
Stigmasta-3,5,22-trien-7-one. MS m/z (rel. int.): 408 (M$^+$, 67), 393 (15), 365 (20), 281 (22), 269 (100), 227 (28), 187 (35), 174 (50), 173 (56), 161 (33), 134 (21).
Stigmasta-3,5-dien-7-one. MS m/z (rel. int.): 410 (M$^+$, 46), 395 (14), 313 (7), 269 (20), 229 (23), 187 (28), 174 (100), 161 (32), 134 (13).

Tables 1 and 2 demonstrate that the subfossil and recent specimens of *Umbilicaria cylindrica* have the same metabolites, whereas the specimens from Canada, Russia, Scotland, Austria, and Switzerland differ from the Greenland material concerning their lichen substances. While the main phenolic compound of subfossil and recent material from Greenland and the Kola Peninsula is gyrophoric acid, this tridepside could not be detected in specimens from Canada, Scotland, Austria, and Switzerland. Remarkable is the stability of gyrophoric acid in the subfossil thalli and the occurrence of the sterol-7-ketones no. 14 - 17 (Tab. 2) and the triterpenes in plants from Greenland only. It seems that the plants from Greenland belong to a separate chemical race. To the best of our knowledge the sterol-7-ketones are new to lichens.

The absence of arabitol and mannitol in the subfossil material is not surprising because these sugar alcohols are easily soluble in water and had been washed out by melted ice.

The subfossil and the recent collections of *U. cylindrica* from Greenland belong to the same chemical race, and chemical changes seem not to have taken place during the long intervening period of ice-cover.

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