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ISOLATION AND IDENTIFICATION OF THE HYPOGLYCEMIC AGENT, CARBOXYATRACTYLATE, FROM *XANTHIUM STRUMARIUM*

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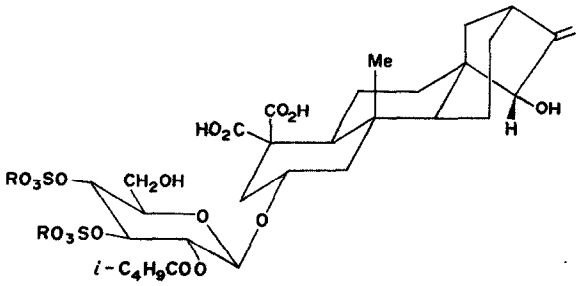
Key Word Index—*Xanthium strumarium*; Compositae; cocklebur; hypoglycemia; potassium carboxyatractylate; carboxyatractyloside.

Plant. Xanthium strumarium burrs (cocklebur). *Source.* Oxford, Mississippi. *Previous work.* Sesquiterpenes [1], essential oils [2], sitosterol, stigmasterol, and campesterol [3,4] and a hypoglycemic agent [5,6]. *Toxicity.* Potassium carboxyatractylate is a very toxic hypoglycemic agent ($LD_{50} = 10.7$ mg/kg; IP in mice) which strongly inhibits translocation of adenine nucleotides across the mitochondrial membrane [7].

Present work. The whole, ground burrs were extracted by successive percolation with hexane, $CHCl_3$, EtOH, and H_2O. The H_2O extract was flash evaporated *in vacuo* at 50°, and the residue was treated with tetrahydrofuran:H_2O; 7:3. This solvent mixture was decanted and removed *in vacuo* at 50°. This residue was triturated with cold MeOH to yield 0.02% of an insoluble glycoside.

The glycoside was purified by forming the potassium salt and recrystallizing from H_2O; ν_{max}^{KBr} 3480, 2980, 1735, 1640, 1270, 1040, 1000, and 800 cm^{-1}. Hydrolysis of the glycoside in aq KOH (20%, reflux for 8 hr.) yielded a sugar, sulfate, isovalarate, and, upon neutralization, an acidic aglycone. Treatment of the aglycone with CH_2N_2 yielded a compound with a MI of *m/e* 392. Subsequent silylation of this derivative yielded a compound with a MI of *m/e* 536.

Direct comparison (mmp, IR, NMR, $[\alpha]_D$, and TCL) of the potassium salt of the glycoside with an authentic sample of potassium carboxyatractylate (**1b**) [8] confirmed the identity of the isolated glycoside as carboxyatractyloside (**1a**).



(1a) R = H
(1b) R = K

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REFERENCES

1. Winters, T. E., Geissman, T. A. and Safir, D. (1969) *J. Org. Chem.* **34,** 153.
2. Baslas, R. K. (1970) *Flavour Ind.* **1,** 474.
3. Mukerje, S. (1970) *Indian J. Pharm.* **32,** 48.
4. Khafagy, S. M. and Metwally, A. M. (1971) *Planta Med.* **19,** 234.
5. Dhar, M. L., Dhar, M. M., Dhawan, B. N., Mehrotra, B. N. and Ray, C. (1968) *Indian J. Exp. Biol.* **6,** 232.
6. Jupiecki, F. P., Ogzewalla, C. D. and Schell, F. M. (1974) *J. Pharm. Sci.* **63,** 1166.
7. Luciani, S., Martini, N. and Santi, R. (1971) *Life Sci.* **10,** 961.
8. Danieli, B., Bombardelli, E., Bonait, A. and Gabetta, B. (1972) *Phytochemistry* **11,** 3501.

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TRITERPENOIDS AND FATTY ACIDS FROM SOME MOSSES. OBTUSIFOLIOL FROM *RACOMITRIUM LANUGINOSUM**

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Key Word Index—Musci; alkanes; tetra- and pentacyclic triterpenoids; obtusifoliol; phytosterols; fatty acids.

Mosses. Anomodon viticulosus Hook. and Tayl; *Brachythecium rivulare* B.S.G.; *Campylopus introflexus* (Hedw.) Brid.; *Ctenidium molluscum* (Hedw.) Mitt.; *Raco-*

*Part 5 in the series "Triterpenes from Mosses". For Part 4 see Marsili, A., Morelli, I., Bernardini, C. and Pacchiani, M. (1972) *Phytochemistry* **11,** 2003.

Table 1. Moss hydrocarbons

Moss	Alkanes									Hopene-b	9(11)-Fernene	Others
	C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}			
B. rivulare	1.0	1.2	1.4		84		5.5			69.5		13.0
Ca. introflexus	2.0	0.8	8.2	1.3	18.9	3.0	33 9	1.4	0 3	20.6		9.6
Ct. molluscum	7 0	1.4	20.9	0 3	9.4		6 3			54.7		
R. lanuginosum	2.2	1 5	9.5	6.1	31.9	2 5	24.1	1.5	20.3			0 4
S. touretii	5 2	1 2	28.1	0 7	12.3	0.8	16.4	0 3	33.1		1 9	

Table 2. Triterpenoid alcohols and sterols

Moss	Ergosterol	Stigmasterol	Campesterol	Sitosterol	Obtusifoliol	Cyclo-eucalenol	31-Norcyclo-laudenol	Cyclo-laudenol	Others
B rivulare	27.6	5.9		57.7		8 7	0 1		
Ca introflexus	20.8	5.5	1.7	29.1		37 7		0.6	4 6
Ct. molluscum	24.2	18.6		54 4					2.8
R lanuginosum		13.8	5 7	2.9	47.8		26.3	3.5	
S. touretii		25.0	4.1	54 2		11 8		0.7	4 2

Table 3. Fatty acids

Fatty acid	Moss*					
	1	2	3	4	5	6
Palmitic	19.7	22.3	8.5	28 7	10.6	25 2
Palmitoleic	2.4	12.7	2.9	5.0	3.0	4.4
Heptadecanoic			0.3		0.5	0.1
Heptadecenoic	1.2	0.7	3.4	0.6	1.0	0.2
Stearic	2.7	1.4	0 2	1.9	0 7	1.2
Oleic	6.4	5.4	6.6	7 5	10 3	5 6
Linoleic + nonadecanoic	32.7	11.5	44.4	17.3	16.7	14.7
Linolenic + arachidic	11.3	11.5	23.3	15.5	50.2	19 7
Nonadecenoic	1.0	1 0	1.5	2.0	1 2	1.0
Gadoleic	4.0	9.0	3.1		2.8	2 3
Eicosadienoic	0.8		1.1	5.3		2 7
Behenic	15 2	16.8	3.9	13.7	3.0	14 4
Lignoceric	2.5	7.7	0.8	2 5		8 5

* Key: 1, *A. viticulosus*; 2, *B. rivulare*; 3, *Ca. introflexus*; 4, *Ct. molluscum*; 5, *R. lanuginosum*; 6, *S. touretii.*

mitrium lanuginosum (Hedw.) Brid.; *Scleropodium touretii* (Brid.) L. J. Koch.

RESULTS

Tables 1–3. Data from GLC analyses directly or on methyl esters in the case of the fatty acids.

EXPERIMENTAL

(For details, see refs. [1] and [2]). Obtusifoliol (identity confirmed by mp, mp of acetate, specific rotation, NMR and MS) was separated from the other alcoholic constituents of *Racomitrium* by chromatography over neutral alumina (eluants, light petrol–Et_2O, 1:1). Analysis of the crude alcoholic fraction by GLC and IR (as done for other mosses) was not suitable for the identification of the compound, since it had the same retention time as sitosterol on two columns, and its IR spectrum was almost indistinguishable from the spectra of the other tetracyclic triterpenic alcohols containing a methylene group in the side chain. It appears possible, therefore, that other mosses may contain obtusifoliol, whose isolation from this moss seems to be in agreement with the view that it is an intermediate in the biotransformation of tetracyclic triterpenes into phytosterols [3]. The presence of hopane derivatives in mosses further confirms the phylogenetic relationship between bryophytes and pteridophytes.

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REFERENCES

1. Marsili, A. and Morelli, I. (1968) *Phytochemistry* **7**, 1705.
2. Marsili, A. and Morelli, I. (1970) *Phytochemistry* **9**, 651.
3. Benveniste, P., Hewlins, M. J. E. and Fritig, B. (1969) *European J. Biochem.* **9**, 526.

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BRASSICASTEROL IN *CLADONIA GONECHA* AND *STEREOCAULON TOMENTOSUM*

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Key Word Index—Lichens; *Cladonia gonecha*; *Stereocaulon tomentosum*; brassicasterol.

Plants. Cladonia gonecha (Ach.) Asah. Cladoniaceae, grown on peat bog; *Stereocaulon tomentosum* Fr., Stereocaulaceae, grown on rock, both from the Agle-Lurudal region. Until recently *Cladonia deformis* (L.) Hoffm. and *C. gonecha* (Ach.) were considered varieties of the same species, *C. deformis*. They are now [1] treated as well