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SESQUITERPENES OF THALLOID LIVERWORTS OF THE GENERA *CONOCEPHALUM, LUNULARIA, METZGERIA* AND *RICCARDIA*

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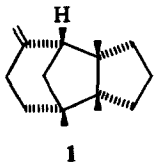
Key Word Index—*Conocephalum*; *Lunularis*; *Metzgeria; Riccardia*; Hepaticae; cadinene; *ent*-sesquiterpenes; δ-cadinene.

Abstract—Thalloid liverworts of orders Metzgeriales and Marchantiales elaborate essential oils distinguishable from those of the Jungermanniales by the absence of β-barbatene and anastreptene. *Riccardia sinuata* elaborates a novel tricyclic exomethylene sesquiterpene of as yet undetermined structure. *Conocephalum conicum* elaborates cadinene-type sesquiterpenes. δ-Cadinene from the latter species is clearly enantiomeric to the same product from vascular plants.

INTRODUCTION

Liverworts have now been shown to produce sesquiterpenes in high yield [1]; moreover liverwort essential oils show several unique features: β-barbatene (**1**) and/or anastreptene (**2**) are found as a constituent of all oils examined thus far [2], and the sesquiterpenes isolated are (when a comparison is possible) strictly enantiomeric to the higher plant product [3, 4]. In the latter the Hepaticae resemble fungi and marine invertibrates [3].

As fruitful as the studies of liverwort essential oils have been with regard to uncovering novel sesquiterpene skeletons, they have not had much chemotaxonomic impact since only a relatively few genera of the leafy liverworts (order Jungermanniales) have been examined. We now present some preliminary results on representatives of two related orders: Metzgeriales and Marchantiales.



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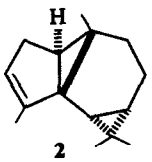

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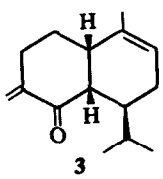

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The only reported data on these thalloid groups is a GLC survey [5] suggesting the production of numerous sesquiterpenes and, in the case of *Conocephalum conicum*, the identification (by IR spectral comparison) of δ-cadinene as a major component [6]. The enantiomeric nature of the product was not determined. There was a particular interest since the one literature structure suggested for a liverwort product, that is not *ent*-germacrene derived, is chiloscyphone (**3**) [4], a cadinene.

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RESULTS AND DISCUSSION

Oil composition data

The oils of *Riccardia sinuata* (Dicks.) Trev. and *Metzgeria conjugata* Lindb. available from earlier studies [5] were re-examined by GLC (see Table 1) using coinjection with authenticated sesquiterpene standards [7]. It was quite clear that neither contained detectable quantities of either β-barbatene or anastreptene. In the case of the oil from *Metzgeria conjugata*, the major product appears to the sensitive and later analyses revealed two quite different compounds. The quantity of oil was not sufficient to allow isolation for characterization.

The major hydrocarbon from *Riccardia sinuata* was collected by preparative GLC. Spectral data (MS parent $m/e = 204.1860$) indicated a tricyclic sesquiterpene structure. The NMR spectrum showed an exomethylene unit, three singlet methyls, and suggested a cyclopropyl ring. The latter was confirmed by an IR absorbance at 3025 cm^{-1}. The spectrum did not correspond to any known compounds (available to us or reported), and we provisionally refer to this substance as sinuene.

Samples of *Lunularia* (*cruciata*?) were collected growing on soil in pots as a weed in the University of Washington greenhouses. The hydrocarbons were isolated in extremely low yield and only analyses on one stationary phase were accomplished. As in *Metzgeria conjugata* the major hydrocarbon is sensitive, decomposing to two new substances. Unfortunately the analyses of the two oils were not done at the same time, nor under comparable conditions, and we cannot be certain that these two substances are identical.

Conocephalum conicum (L.) Lindb. is a circumboreal species which has a noted resistance to insects and snails thought to be associated with the oily exudate produced [8]. Four different batches of this liverwort were collected in Washington State and the essential oils were isolated either by direct steam distillation or by steam distillation of the ether extracts. Essential oils were isolated in 0.02–0.2% yield based on fresh weight and contained

10–15% sesquiterpene olefins (eluted with pentane from alumina). The GLC analyses of the hydrocarbon fractions are summarized in Table 1. The considerable variation may be associated, in part, with the difficulties inherent in obtaining pure liverwort samples free of soil. Only in the case of δ-cadinene was sufficient material collected for a full characterization (NMR, IR, GLC coinjection, and rotatory comparison). The material obtained, displayed CD data which was mirror image related to that observed for the higher plant product (Cade oil, *Juniperus* sp., in this case):

(+)-δ-cadinene (Cade oil)—$\Delta\varepsilon_{212} = -3.7$, $\Delta\varepsilon_{193} = +10.6$.

(−)-δ-cadinene (*C. conicum*)—$\Delta\varepsilon_{213} = +2.6$, $\Delta\varepsilon_{190} = -11$.

The apparent error in $\Delta\varepsilon_{212}$ is likely the result of high noise levels on such determinations on dilute solution. The amplitude of a CD couplet is a better measure of optical purity.

Radiolabel incorporation experiments

C. conicum plant material whole, homogenized, and as shreds in water, water containing DMSO, and in mineral nutrient media (see Experimental) were treated with 5-^{3}H-MVA and/or 2-^{14}C-acetate for 3 hr to 4 days. Each incubation mixture was then frozen (−30°) and the material steam distilled, the distillate being separated into ether and water-soluble fractions. Only 0.08–2% of the added radioactivity ended up in the ether extractable essential oil. Further purification was effected by column chromatography on basic alumina. The hydrocarbon fractions (eluted with hexane) contained from < 0.0008–0.01% of the added radioactivity (resulting levels barely above background levels). The benzene eluted fractions were also essentially free of tracer. Ethyl acetate and methanol eluted fractions contained *ca* 0.1% of the tracer. In most cases only 10–15% of the ether extracted tracer could be eluted from the column. Apparently little or no tracer is incorporated into sesquiterpene hydrocarbons and related oxygenated materials.

DISCUSSION

These preliminary results suggest that thalloid liverworts (orders Metzgeriales and Marchantiales) are distinguishable from the Jungermanniales by the absence of β-barbatene and anastreptene. The essential oils obtained were simpler than those of the leafy liverworts but we have not been able to obtain sufficient material to fully characterize the novel sesquiterpene constituents, in part due to their lability. The report of δ-cadinene as a constituent of *C. conicum* [6] is now fully confirmed and the presence of other cadalenes—10-epizonarene, calamenene (**3**) and calcorene (**4**)—is suggested from the GLC analysis. The major constituent, (−)-δ-cadinene (**6**), is clearly *ent*-germacrene derived from CD comparison with the *Juniperus* derived material. With this determination the occurrence of the major germacrene-related sesquiterpene groups in liverworts is established. All fully correlated materials are enantiomeric to the products from vascular plants. The one exception to this rule [4] warrants reinvestigation.

Table 1. GLC analyses of the hydrocarbon fractions of thalloid liverwort essential oils*

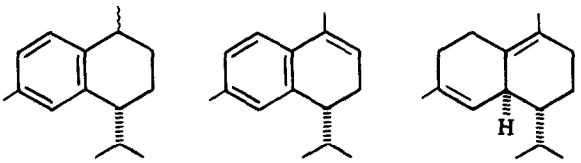
Liverwort specimen	pk. #	%	I_A^{190}	I_C^{150}	I_C^{165}	Correspondence
Riccardia sinuata	1	84	1482	1646		'sinuene'
	2	3	1523	1702		
	3	3	1564	1761		
	4	2	1564?	1835		(calamenene or
	5	5	1697	?		cuparene?)
Metzgeria conjugata						
(initial assays)	1†		1498	1546		
	(after 1 mo. storage)					
	2	60–70	1527	(I_D^{160} = 1743)		
	3	20–30	1554	(I_D = 1791)		
Lunularia (cruciata?)	1	60–10‡			~1516	
	1b	0–20‡			1555	
	1c	0–40‡			1624	
	2	5–10			1652	
Conocephalum conicum	2	1–5	1396		1526	
	5	7–10	1490	1630	(1630)	
	6	2	1500	—	—	
	7	2–19	1519	~1730		
	9	2–9	1537	(~1735)		(10-epi-zonarene)§
	11	15–50	1562	1771	1786	δ-cadinene
	12	5–10	1565	1832	(1832)	(calamenene?)
	14	5–15	1585		1930	(α-calcorene)§
	17	8–20	1677			

* Stationary phase code: A, Apiezon-L; C, Carbowax-20M; D, Diethyleneglycol succinate (DEGS).

† Unstable: apparently rearranges to components designated *M. conjugata* #2 and #3.

‡ *Lunularia* component #1 appears unstable. The % composition varies with time, with peaks #1b and #1c growing at the expense of #1.

§ By comparison of index values only: see ref. [12].



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Preliminary attempts at tracer studies of liverwort sesquiterpenes biosynthesis have been unsuccessful, but efforts in this direction are continuing.

EXPERIMENTAL

Analytic and preparative GLC was carried out as in refs. [2, 7]. Rotatory data and CD spectra were recorded as *ca* 0.5–10 mM solns in pentane (l. = 0.1–10 mm). Prior to determination of CD and rotatory constants olefins were purified by chromatographic filtration through Woelm Basic Al_2O_3 (Act. I) using spectrograde pentane. Other spectral data is obtained directly on material isolated by preparative GLC. NMR spectra are at 60 MHz unless otherwise indicated with TMS as an internal standard (δ = 0.00 ppm).

Essential oil isolation. Essential oils of *Metzgeria conjugata* and *Riccardia sinuata* were available from previous studies [5] and voucher specimens are in the herbarium of S.H. The oil samples, stored in sealed ampoules, were dissolved in *n*-decane for direct GC analysis (see Table 1). The major hydrocarbon from *Riccardia sinuata* collected from an Apiezon-L column at 190° displayed the following spectral data: $[\alpha]_D$ = + 5°, $[\alpha]_{300}$ = + 69° $\Delta\varepsilon_{199}$ = + 4.7. $\Delta\varepsilon_{185}$ = − 1.7!; δ_{CCl_4} 4.78 (2H, C = CH_2, $W_{h/2}$ ~ 8 Hz), 2.1–2.4 (~ 1H, allylic), 1.07, 0.96, and 0.74 (three 3H *s*., Me's), and 0.3–0.6 ppm (2–3H?, cyclopropyl-H); IR (film) 3080, 1652, 888 (C=CH_2), 3025 (cyclopropyl C—H), 1383 cm^{-1} (sharp, not doublet, no CMe?); MS: *m/e* (% abundance, composition, mmass error)—204.1860 (7, $C_{15}H_{24}$ − 1.6), 189.1638 (6, $C_{14}H_{21}$ −0.4), 137.1336 (15, $C_{10}H_{17}$ + 0.8), 111.1176 (31, C_8H_{15} + 0.4), and 43.0528 amu (100%, C_3H_7 − 2.0 mmass).

Lunularia (cruciata?). Collected in the University of Washington greenhouses (2/72) [9]. Steam distillation with added $CaCO_3$ (1 g per 100 g fr. liverwort wt) and 50 ml glycol afforded a 0.02% yield (fr. wt basis) of Et_2O soluble essential oil which was subjected to direct GC analysis (Table 1).

Conocephalum conicum. Collected at Granite Falls, Washington State, on 9/13/72 and 10/14/73 [10]. The plant material was freed of mosses by hand sorting and washed of soil. Each collection was separately steam distilled as above. On 200–500 g (fr. wt) batches the yield of oil was typically 0.05%. Chromatography on Al_2O_3 (Woelm, Act I, neutral) affords a 0.005% yield of hydrocarbons (eluted with hexane, for range of GLC analyses see Table 1); elution with EtOAc affords 0.02–0.03% of polar materials having a distinct forest floor aroma. Preparative GLC (Carbowax, 170°) afforded a pure sample (GLC on two phases) of the major hydrocarbon, identified as (−)-δ-cadinene based on spectral data: $[\alpha]_D$ = − 60 ± 10°; $\Delta\varepsilon_{213}$ = + 2.6 ± 0.6, $\Delta\varepsilon_{190}$ = − 11 ± 2; δ_{CCl_4} 5.35 (vinyl-H), 1.62 (vinyl-Me), 0.95 and 0.76 ppm (2 Me *d*, *J* = 6.5, 7.5 Hz)—reported for the enantiomer $[\alpha]_D$ = + 90 ($CHCl_3$); [11] δ_{CCl_4} 5.34, 1.62, 0.95 *d*, 0.79 d. [12]. A sample of (+)-δ-cadinene was isolated from Cade oil [12] giving $[\alpha]_D$ = + 64° and the CD data given in the Results.

Radiotracer incorporation studies. Ca 10 g plant material (*C. conicum*, completely free of soil) was used for each tracer biosynthetic experiment. The added tracers in each case totalled 10^7 dpm (^3H, ^{14}C, or ^3H + ^{14}C) and were 2-^{14}C-acetate (0.63 mc/mg) and (±)-mevalonic-5-^3H-acid DBED salt (0.83 mc/mg). The plants were grown in H_2O under ordinary laboratory lighting for 4 days prior to the incubation experiment. Incubations were done with whole plant, *ca* 2 × 10 mm plant shreds, and with more thoroughly crushed samples (Waring blender). The media were: H_2O, 2% aq. DMSO, and mineral media [1 mM KH_2PO_4, 5 mM KNO_3 and $Ca(NO_3)_2$, 2 mM $Mg(SO_4)_2$]. Incubation periods varied from one to 4 days. The disappointing results are summarized in the Results. The only additional point of interest being that the best incorporation into the hydrocarbon fraction occurred using shreds of the plant with a 4 day incubation period using mineral media with normal laboratory lighting. Acetate was incorporated more efficiently. But in no case was the %-incorporation above 0.012. One attempt to treat the hydrocarbon fraction with HCl and isolate labelled cadinene dihydrochloride by co-chromatography and co-crystallization with authentic material failed to yield levels above background. This is likely due to the use of dihydrochloride sample derived from (+)-cadinenes.

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