Vinyl-Olefines and Sesquiterpenes in the Root-Oil of *Senecio isatideus*

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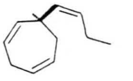
Dedicated to Prof. Ferdinand Bohlmann *on Occasion of His 60th Birthday*

Vinyl-olefines, 1,7,10,13-Hexadecatetraene, Sesquiterpenes, *Senecio isatideus* (Compositae), Mass Spectroscopic Determination of double bond positions

A series of polyunsaturated vinyl-hydrocarbons (n-C_{15} to n-C_{17}) (10, 11, 12, 15, 16 and 17) including the as yet unknown *all-cis*-1,7,10,13-hexadecatetraene (15) as well as some rare sesquiterpenes such as isocomene (8) or modhephene (7) have been isolated from the root oil of *Senecio isatideus.* Unambigous positioning of the double bonds of the polyolefines was easily established by ^{13}C-NMR spectroscopy [16]. A new mass spectroscopical method utilizing NO as a reactant gas for chemical ionization [1] made possible the reliable identification of minor and trace constituents without any chemical pretreatment or further derivatisation.

Introduction

Senecio isatideus, a South African representative of the ubiquitous class Senecioninae (Compositae) has the peculiar property to produce in its green parts large amounts of ectocarpene (18) [2], which was recognized earlier as the chemical messenger of female gametes of the sea-weed *Ectocarpus siliculosus* [3, 4].



(+)-(1 S)-1-(*cis*-1-butenyl)-2,5-cycloheptadiene (ectocarpene) (18)

Since, obviously, terrestrial plants are more easily obtained than the gametes of brown algae, the discovery of the algal sex-attractant in a composite, that can be grown abundantly, offered us a far superior biological system to study ectocarpene's biosynthesis as well as its biodegradation.

It has been established that some of the better examined species of brown algae, such as *Fucus* [5], *Ectocarpus* [6] or *Laminaria* [7], not only use highly unsaturated open-chain or alicyclic vinyl-hydrocarbons [8] as chemical signals in their reproductive cycle, but contain also other long-chain vinyl-polyolefines in their thalli [9, 10]. Whether this occurrence may be interrelated and represents a parallel enzymatic mechanism of vinyl group or even precursor formation is under current investigation. These observations together with the fact that ectocarpene (18) is only one member of a whole family of C_{11}-hydrocarbons found in algae [11, 12] encouraged us to look more carefully into the hydrocarbon inventory of *Senecio isatideus.* Indeed, its root oil contains appreciable amounts of expected and unexpected vinyl-hydrocarbons, as listed in Table I.

Materials and Methods

Senecio isatideus DC (77/101, Natal) was cultivated from seeds provided generously by Dr. O. Hilliard (Department of Botany, The University of Natal, Pietermaritzburg, SA). Only flowering plants were used for extraction after it had been found that at this time ectocarpene production is highest.

Extraction procedure

350 g roots of freshly harvested plants were carefully washed, chopped and ground in a Waring Blendor with 300 ml distilled water. The mash was extracted under stirring for 24 h with 200 ml distilled pentane, then filtered by suction and the filter cake washed with additional 100 ml pentane. The combined pentane phases were dried over $MgSO_4$

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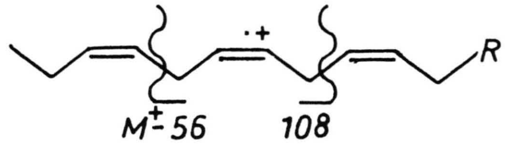
and concentrated under vacuum. The crude oily residue (root oil) was further fractionated and purified by column chromatography on silica gel using pentane as the eluant. By continous GLC monitoring two hydrocarbon fractions were separated, the first containing the saturated to dienoic compounds, the second representing the more polar trienes and tetraenes (comp. Fig. 1 and Table I). The total amount of hydrocarbons recovered was 0.35 g, corresponding to 0.1% of fresh plant material. An aliquot was further chromatographed on a column of silica gel coated with 10% $AgNO_3$ yielding pure samples of the major compounds (8), (16) and (17).

Gaschromatography and determination of Kovàts indices [13]

All GLC analyses were performed on a Carlo Erba gaschromatograph Fractovap 2900 equipped with a plotting integrator (Hewlett-Packard 3390A). The columns were Duran glass capillaries 50 m × 0.4 mm coated with OV 101 or Ucon 75 h 90000. Kovàts indices were determined by co-injection of a suitable *n*-alkane pattern together with the sample at a properly selected isothermal temperature level. As far as available authentic samples (see reference cited) were used to establish identity of compounds (indices given in brackets).

NMR- and mass-spectrometry

^{1}H-NMR spectra were recorded on a Varian NMR-spectrometer EM 390 90 MHz; ^{13}C-NMR spectra were run on a CFT 20. These analyses, however, could only be made with the purified products (8), (16) and (17) [16]. Final information on the nature of minor and even trace constituents came from a new mass spectroscopic technique which uses NO gas as the reactant for mild and selective ionization of double bonds [14]. This very effective method proved of special value for compounds with a "linolenic" pattern of homo-conjugated double bonds within the molecule. The observed fragmentation pattern may be plausibly explained by the following mechanism [1].



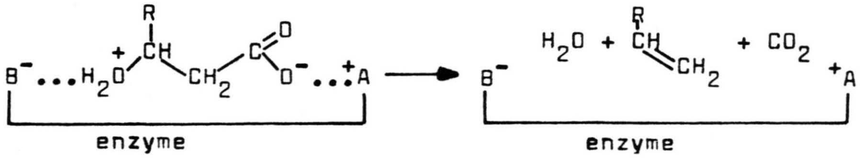
All compounds examined possessing a "linolenic" arrangement of double bonds gave the same ion $m/z = 108$ as base peak. This may be due to an attack by NO^+ on the middle most double bond, followed by a vinylic cleavage on either side and accompanying loss of HNO. This leads in the case of (16) to the fragment ions $m/z = 108$ and $m/z = 176$. Consequently, the new hydrocarbon (15) shows $m/z = 108$ as base peak, too, and a second prominent fragment at $m/z = 162$ which indicates an identical overal structure with only one $-CH_2$-group less in R. Additionally synthetic samples [16] were treated the same way and gave congruent spectra.

Results and Discussion

The root-oil of *Senecio isatideus* contains about 0.1% of a mixture of linear and alicyclic hydrocarbons. Major constituents are the unbranched C_{17}-vinyl-hydrocarbons (75% of total amount) among which *all-cis*-1,8,11,14-heptadecatetraene (16) [15, 16] is by far the most abundant (88% of C_{17}-olefines), followed by the corresponding triene (17) (7%), diene (11) (4%) and the monoene (12), which is found as a trace constituent only (1%).

In the C_{15}-hydrocarbon fraction unbranched hydrocarbon chains are almost nil, and sesquiterpenes are dominating. Again one major product (13% of total weight) was isolated and identified as the tricyclic sesquiterpene isocomene (8), whose occurrence in Compositae and structure has been established by Bohlmann [17] and Zalkow [18]. All other olefines and saturated hydrocarbons contribute to the total only 0.5 to 2% for each individual compound. Table I and Fig. 1 give a complete survey of all compounds isolated and identified.

It will be evident that chain olefines derive biogenetically from fatty acids since they are unbranched and have similar position of the double bonds, particularly in most cases the *ω-cis*-butenyl end. Bohlmann [20] has postulated a route to vinyl-groups in plant hydrocarbons making use of a heterolytic fragmentation of a correspondingly functionalized *β*-hydroxy fatty acid. Such hydroxy acids are easily available from the normal *β*-oxidation of fatty acids. Simple acid/base catalysis on an appropriately binding enzyme surface would suffice to carry out fragmentation of this substrate into alkene, H_2O and CO_2.



Furthermore we may point to the occurrence of the even numbered *all-cis*-1,7,10,13-hexadecatetraene (15), which hitherto has not been isolated from a natural source. It conceivably is formed by α-oxidation – a reaction widespread in Compositae – of linolenic acid followed by the above sequence of reactions.

Finally it is to be noted that the highly unsaturated polyenes alone are formed in appreciable amounts, whereas the dienes and monoenes are found as minor or trace constituents only. On the other hand, among the assumed precursor fatty acids, linolenic acid is present in root lipids of *Senecio isatideus* but in traces (1%), whereas linoleic acid (88%) and oleic acid (10% of total C_{18}-fatty acids) represent the major components of the C_{18}-family. This observation may indicate that the number of double bonds determines eventually the metabolic fate of a given fatty acid. The more highly unsaturated its distal end, the better available is its carboxylic end to the fragmentation reactions envisioned, either by stronger binding or

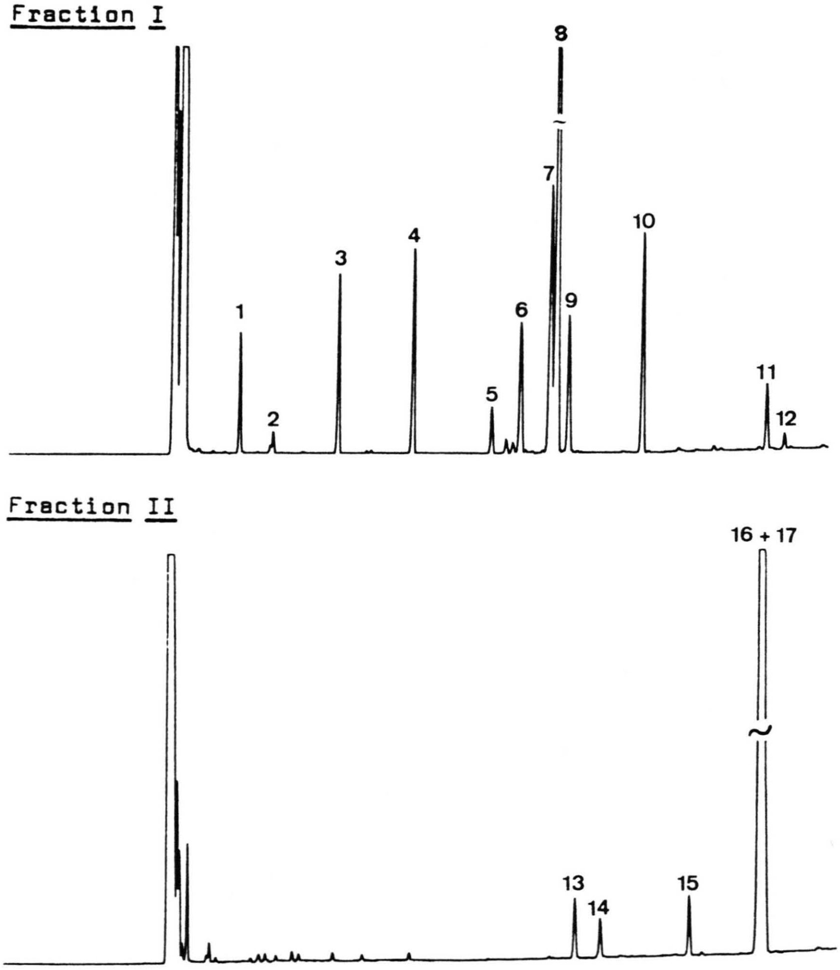
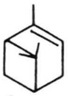
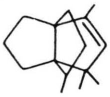
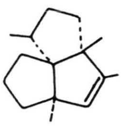
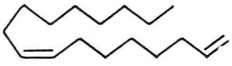
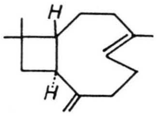
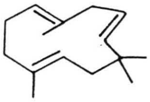
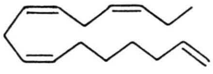
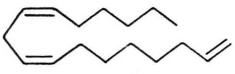


Fig. 1. Gaschromatographic separation of hydrocarbons from the root-oil of *Senecio isatideus*. Column used: 50 m × 0.4 mm Duranglass capillary coated with OV 101. Conditions: Temperature programmed elution ranging from 60–210 °C; rate: 10 °C/min. All identified compound are numbered according to their elution order and are given in Table I.

Table I. Hydrocarbons from the root oil of *Senecio isatideus.*

Peak-number	Compound (formula)	Kovàts-index		MS (CI-NO) *m/z* (%-intens.)	other methods	ref.
		OV 101	UCON 75 H			
1		947.4 (947.4)	1004.1 (1003.8)		a	
2	decane	1000.4 (1000.0)	1000.7 (1000.0)		a	
3	undecane	1100.5 (1100.0)	1100.2 (1100.0)		a	
4	dodecane	1200.2 (1200.0)	1200.7 (1200.0)		a	
5	tridecane	1300.2 (1300.0)	1300.7 (1300.0)		a	
6	sesquiterpene (unidentified)	1357.9	1466.0		a	
7		1408.0	1508.3		a	[21]
8		1413.8	1519.2		a, b, d	[17, 18]
9	sesquiterpene (unidentified)	1435	1552.7		a	
10	1-pentadecene	1489.8 (1490.2)	1533.5 (1533.3)		a	[19]
11		1667.6 (1668.0)	1745.9 (1745.5)	266 (13), 236 (100), 218 (5), 148/146 (10, 14), 123/124 (27), 110 (60), 96 (63), 81 (51), 67 (38)	a	[16, 19]
12	1-heptadecene	1690.8 (1690.3)	1734.8 (1734.4)		a	[19]
13		1447.8 (1447.8)	1577.0 (1576.4)		a	
14		1478.2 (1478.2)	1634.9 (1634.4)		a	
15		1565.1 (1565.3)	1722.8 (1722.7)	248 (1), 218 (9), 189 (5), 180 (10), **162 (28),** 146 (9), 121/122/126 (13/12/13), **108 (100),** 94 (10), 80 (19)	a	[16]
16		1662.4 (1662.4)	1822.2 (1821.6)	262 (4), 232 (12), 194 (11), **176 (23),** 136 (6), 126 (17), **108 (100),** 94/95 (5/5), 79/80 (4/4), 67 (3)	a, b, c, d	[2, 15, 16]
17			1774.5 (1774.5)	264 (4), 234 (13), 163 (8), 149 (12), 135/138/140 (3/7/4), 124 (14), 109/110 (100/95), 95 (7), 84 (31)	a, b, c	[16, 19]

Other methods: a) mass spectr. (EI 70 eV), b) ^{1}H-NMR, c) ^{13}C-NMR, d) IR.

better positioning. The same is true in the C_{15}-hydrocarbon group, where only traces of 1-pentadecene (10) contribute to the family of vinyl-hydrocarbons though palmitic acid is present in abundance. Further studies with isolated systems and suitable synthetic precursors are needed to eludicate these biogenetic steps and their overall control in metabolism.

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