

New Cembranoids from Tobacco, II*

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Z. Naturforsch. **38c**, 515–516 (1983); received March 17, 1983

Nicotiana tabacum, Solanaceae, Diterpenoids, Cembranoids, Methylethers

The methylethers 4-O,8-O-dimethyl-(1*S*, 2*E*, 4*R*, 6*E*, 8*S*, 11*E*)-2,6,11-cembratriene-4,8-diol (**9**), 4-O-methyl-(1*S*, 2*E*, 4*R*, 7*E*, 11*E*)-2,7,11-cembratriene-4,6-diol (**4**) und 4-O,6-O-methyl-(1*S*, 2*E*, 4*R*, 7*E*, 11*E*)-2,7,11-cembratriene-4,6-diol (**8**) were identified as new natural products from tobacco. They were isolated from processed tobacco as well as from leaf surface gum of fresh tobacco.

The spectral data of all six methylethers of (1*S*, 2*E*, 4*S*, 6*R*, 7*E*, 11*E*)- and (1*S*, 2*E*, 4*R*, 6*R*, 7*E*, 11*E*)-2,7,11-cembratrienen-4,6-diol are given.

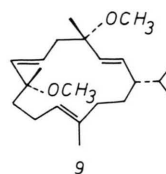
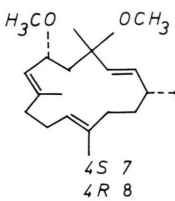
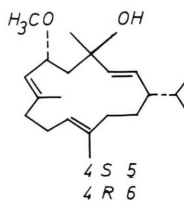
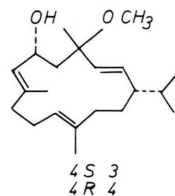
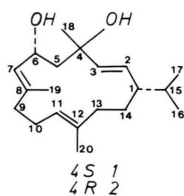
Leaves of *Nicotiana tabacum* are covered with a sticky exudate, which contains diterpenoids. Depending on the genetic background, tobacco cultivars produce the macrocyclic cembranoids, the carbocyclic labdanoids or both [1, 2]. Bioconversion and biodegradation of these compounds leads to a large number of diterpenoid-derivatives, and norterpeneoids which are important tobacco flavour substances [3]. To get more insight into mechanisms of bioconversion and biodegradation, the distribution of diterpenoids in leaf surface gum and aged tobacco was investigated.

Aged tobacco was extracted with liquid CO₂ and fractionated as described earlier [4]. The fractions eluted from silicagel with petrolether:diethylether 75:25 and 50:50 (v:v) were separated further by repeated columnchromatography and preparative TLC. Some of the isolates were tentatively identified as methylethers of cembranoids by molecular ion, by fragment ion $M^+ - 32$ ($M^+ - \text{CH}_3\text{OH}$) and by singlets between 3.1 and 3.25 ppm in ¹H-NMR spectra. Investigation of MS, H-NMR ¹³C-NMR data resulted in identification of a momethylether [5] and a dimethylether [6] as derivatives of 2-7,11-cembratriene-4,6-diols.

As far as we know methylethers of cembranoids have not been described as natural occurring compounds. For precise stereochemical assignment all

possible methylethers of the two natural occurring (1*S*, 2*E*, 4*S*, 6*R*, 7*E*, 11*E*)- and (1*S*, 2*E*, 4*R*, 6*R*, 7*E*, 11*E*)-2,7,11-cembratriene-4,6 diols (**1**, **2**) were synthesized by Williamson-Synthesis with methyljodide and sodiumhydride in dry diethylether or tetrahydrofurane at moderate temperature.

Comparison of spectral generated from natural occurring compounds and synthesized products



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* Part I: see [4].

0341-0382/83/0700-0515 \$ 01.30/0



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Table I. ^{13}C -NMR chemical shifts and assignments for compounds 3–9^a.

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11
3	46.7	131.0	133.3	76.9	48.5	66.4	130.4	136.2	39.1	23.4	124.6
4	46.5	131.5	134.0	75.7	53.0	63.9	133.1	135.6	38.7	23.0	124.3
5	46.4	127.4	137.7	72.2	51.7	74.7	128.4	137.9	38.7	23.3	124.4
6	46.1	129.2	136.4	71.1	50.7	72.8	130.0	137.6	38.8	23.0	124.4
7	46.6	129.7	133.6	76.7	46.5	74.3	128.9	138.0	38.9	23.2	124.4
8	46.4	129.5	134.4	75.4	51.0	72.5	132.8	137.1	38.8	23.0	124.4
9	47.6	127.9	135.9	76.9	47.3	132.7	138.2	77.9	42.2	27.9	126.5
	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20	C-21	C-22
3	133.6	36.9	27.7	37.8	20.6 ^b	19.4 ^b	25.0	15.8	15.0	49.8	
4	132.8	36.4	27.9	33.3	20.7 ^b	19.3 ^b	22.2	16.0	15.1	50.2	
5	133.1	36.7	27.9	33.0	20.6 ^b	19.3 ^b	29.6	16.0	14.7	55.0	
6	132.8	36.4	27.6	32.9	20.5 ^b	19.3 ^b	28.6	15.8	14.8	54.9	
7	133.5	36.7	27.8	32.8	20.7 ^b	19.4 ^b	26.6	15.7	14.8	49.9	54.9
8	132.8	36.4	27.9	33.4	20.7 ^b	19.3 ^b	22.1	16.0	14.9	50.2	54.0
9	131.0	37.0	22.2	33.0	21.1 ^b	20.9 ^b	20.1 ^b	19.9 ^b	13.9	49.8	50.4

^a ρ -Values in CDCl_3 .^b May be reversed.

(Table I) led to structure elucidation of 4-O-methyl-(1*S*, 2*E*, 4*R*, 6*E*, 11*E*)-2,7,11-cembratriene-4,6-diol (**4**) and 4-O,6-O-dimethyl-(1*S*, 2*E*, 4*R*, 6*R*, 7*E*, 11*E*)-2,7,11-cembratriene-4,6-diol (**8**).

In addition the structure of another natural occurring dimethylether of 2,6,11-cembratriene-4,8-diol (**9**) was clearly proved by MS, ^1H -NMR and ^{13}C -NMR [7]. The precise stereochemistry was elucidated by comparison of spectral data from product generated by etherification of (1*S*, 2*E*, 4*R*, 6*E*, 8*S*, 11*E*)-2,6,11-cembratriene-4,8-diol [8] and isolated tobacco constituent.

A first comparison of the distribution in different tobacco types showed that methylethers of cembranoids are enriched in Burley and Oriental tobacco, while only minor amounts could be detected in Virginia tobacco; whether this is due to genetic attributes or to different curing methods cannot yet be decided.

Leaf surface gum of fresh tobacco, which was prepared and fractionated as described earlier [9] contained compounds **4** and **8** in various quantities.

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- [2] W. W. Reid, *Ann. Tabac S.E.I.T.A.* **2**, 145 (1974).
- [3] C. R. Enzell, I. Wahlberg, and A. J. Aasen, *Progress Chem. Natural Products* **34**, 1 (1977).
- [4] U. Brümmer, C. Paulsen, G. Spremberg, F. Seehofer, and V. Heemann, *Z. Naturforsch.* **36c**, 1077 (1981).
- [5] MS (70 eV) [m/z (%): 320 (M^+ , 0.3), 302 ($\text{M}^+ - \text{H}_2\text{O}$, 7), 288 ($\text{M}^+ - \text{CH}_3\text{OH}$, 6), 270(39), 255(14), 227(37), 187(18), 159(37), 145(38), 133(36), 119(44), 105(62), 93(64), 85(100), 81(82). ^1H -NMR (CDCl_3): $\rho = 0.77$ (d, $J = 6.9$ Hz)/0.80 (d, $J = 6.8$ Hz) (H-16/H-17), 1.22 (s, H-18), 1.46 (s, H-20), 1.64 (d, $J = 1.2$ Hz) (H-19), 1.77 ($J = 8.6$ Hz and $J = -14.4$ Hz) (H-5_A), 2.08 (d, $J = 1.2$ Hz and $J = 14.4$ Hz) (H-5_B), 3.12 (s, H-21), 4.83 (ddd, $J = 1.2$, 8.6 and 10.0 Hz, H-6), 4.95 (broad t, H-11), 5.18 (d, $J = 15.8$ Hz), 5.21 (dd, $J = 15.8$ and 6.3 Hz), 5.27 (dd, $J = 10.0$ and 1.2 Hz, H-7).
- [6] MS (70 eV) [m/z (%): 334 (M^+ , 0.7), 319 ($\text{M}^+ - \text{CH}_3$, 0.5), 302 ($\text{M}^+ - \text{CH}_3\text{OH}$, 12), 287 (1.8), 270 (21), 255(13), 227(24), 153(22), 105(50), 85(100), 81(68). ^1H -NMR (CDCl_3): $\delta = 0.82$ (d, $J = 6.8$ Hz)/0.86 (d, $J = 6.9$ Hz) (H-16/H-17), 1.23 (s, H-18), 1.49 (s, H-20), 1.67 (d, $J = 1.0$ Hz, H-19), 1.71 (dd, $J = 8.0$ and -14.0 Hz, H-5_A), 2.11 (dd, $J = 1.0$ and -14.0 Hz, H-5_B), 3.15 (s, H-21), 3.23 (s, H-22), 4.27 (ddd, $J = 1.2$ and 8.0 and 10.0 Hz, H-6), 5.02 (broad t, H-11), 5.05 (dd, $J = 1.0$ and 10 Hz, H-7), 5.12 (d, $J = 15.8$ Hz, H-3), 5.22 (dd, $J = 4.8$ and 15.8 Hz, H-2).
- [7] MS (70 eV) [m/z (%): 334 (M^+ , 0.2), 302 ($\text{M}^+ - \text{CH}_3\text{OH}$, 7), 287 (302- CH_3 , 2), 270(66), 255(30), 227(58), 199(14), 185(28), 171(32), 159(40), 145 (64), 131(52), 119(66), 105(92), 91(94), 85(80), 81(100), 79(92), 55(90). ^1H -NMR (CDCl_3): $\delta = 0.82$ (d, $J = 6.8$ Hz)/0.83 (d, $J = 6.8$ Hz) (H-16/H-17), 1.29 (ss, H-18/H-19), 1.47 (s, H-20), 2.12 (dd, $J = 10.5$ and 14.4 Hz, H-5_A), 2.63 (ddd, $J = 2.0$, 2.7 and -14.4 Hz, H-5_B), 3.13 (s, H-21), 3.14 (s, H-22), 5.21 (dd, $J = 8.8$ and 15.7 Hz, H-2), 5.26 (d, $J = 15.7$ Hz, H-3), 5.33 (broad t, H-11), 5.47 (dd, $J = 2.0$ and 16.0 Hz, H-7), 5.72 (ddd, $J = 2.7$, 10.5 and 16.0 Hz, H-6).
- [8] I. Wahlberg, D. Behr, A.-U. Eklund, T. Nishida, C. Enzell, and J.-E. Berg, *Acta Chem. Scand.* **B36**, 443 (1982).
- [9] V. Heemann, U. Brümmer, G. Spremberg, and F. Seehofer, *Beiträge z. Tabakforschung* **11**, 107 (1981).