New Cembranoids from Burley Tobacco

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The liquid CO_2-extract of aged burley tobacco was investigated for its content of diterpenoids, which are considered as important precursors of flavour constituents. Among several cembranoids, already known from various tobacco types, 4,8-dimethyl-8-hydroxy-11-(1-methyl-ethyl)-14-oxo-pentadeca-5,9-diene-4-olide, a new natural product, could be identified, evidently originating from 2,6,11-cembratriene-4,8-diol by oxidative cleavage of the 11,12-double bond with subsequent cyclization to the *seco*-lactone **14**.

Leaves of *Nicotiana tabacum* are covered with a sticky execude, which contains diterpenoids. Depending on the genetic background, tobacco cultivars produce the macrocyclic cembranoids, the carbocyclic labdanoids or both [1, 2]. Biodegradation of these compounds leads to a large number of nor-diterpenoids, which are considered to be important tobacco flavours [3]. To get more insight into these degradation mechanisms, the distribution of diterpenoids in aged burley tobacco was investigated.

Extraction of 30 kg tobacco with liquid CO_2 yielded about 450 g of an extract, containing 44% H_2O. High-vacuum destillation of the organic layer produced 95 g of a concentrate, with 70% nicotine and 10% neophytadiene as the main constituents. The destillate was further separated by column chromatography, using silicagel. A fraction, enriched with diterpenoids, eluted with 100% diethylether and was subjected to repeated column chromatography, TLC and HPLC, using columns packed with Partisil-10 PAC (Whatman).

In addition to the (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**) [4] and (1 S, 2 E, 4 S, 6 E, 8 R, 11 S)- and (1 S, 2 E, 4 R, 6 E, 8 R, 11 S)-8,11-epoxy-2,6,12(20)-cembratriene-4-ols (**3**, **4**) [5], already found in various tobacco types, several compounds new in burley respectivley in tobacco could be identified.

Three of the four possible diastereomers of 2,6,11-cembratriene-4,8-diol ($\mathbf{5}_{1-3}$) were isolated and the structure proved by MS, ^{1}H-NMR and ^{13}C-NMR (Table I). α- and β-2,6,11-cembratriene-4,8-diol (**5**) were already identified by Rowland *et al.* [6] without precise determination of the stereochemistry of both compounds. The correlation to the α/β nomenclature was based on α- and β-2,7,11-cembratriene-4,6-diol (**1**, **2**). The present spectral data failed either to relate compound $\mathbf{5}_1$, $\mathbf{5}_2$ or $\mathbf{5}_3$ to α- and β-2,6,11-cembratriene-4,8-diol, as published by Rowland *et al.* [6] or to determine the absolute configuration.

Therefore $\mathbf{5}_{1-3}$ were subjected to an x-ray-analysis, the results of which are reported elsewhere.

The two isomeres of 2,7,11-cembratriene-4-ol (**6**, **7**) were isolated for the first time from Virgin tobacco [2]. The stereochemical assignment was achieved by Wahlberg *et al.* [8] to be (1 S, 2 E, 4 S, 7 E, 11 E)- and (1 S, 2 E, 4 R, 7 E, 11 E)-2, 7,11-cembratriene-4-ol. The spectral properties of both diastereomeres, now isolated from burley were in good accordance with the published data, the quantitative ratio for compound **6** and **7** for the investigated burley being 2:1, as calculated by GC.

8,11-Epoxy-2,6-cembradiene-4,12-diol (**8**) and 11,12-epoxy-2,7-cembradiene-4,6-diol (**9**), isolated now from burley, showed the same spectral data (MS, ^{1}H-NMR, ^{13}C-NMR), as published by Behr *et al.* [9, 10] for (1 S, 2 E, 4 S, 6 E, 8 R, 11 S, 12 R)-8,11-epoxy-2,6-cembradiene-4,12-diol and (1 S, 2 E, 4 R, 7 E, 11 S, 12 S)-11,12-epoxy-2,6-cembradiene-4,6-diol, both recently identified from Greek tobacco.

The identification of the ketol (**10**), isolated formerly from smoke-cured tobacco [11], is based on comparison with an authentic sample, prepared by CrO_3-oxydation of **1**. The spectra are identical with those, published by Roberts and Rowland [4].

The *seco*-diketone (**11**) also found in smoke-cured tobacco [11] and the *seco*-aldehyde (**12**), isolated recently from Virgin tobacco, were tentatively identified by their MS and ^{1}H-NMR features and the structures verified by ^{13}C-NMR (Table I). The

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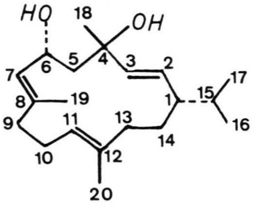
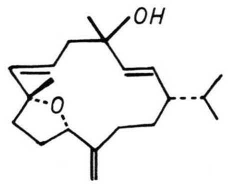


4S **1**
4R **2**



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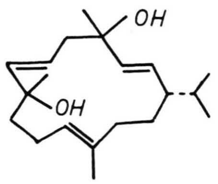

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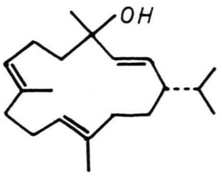
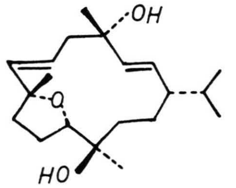


4S 6
4R 7



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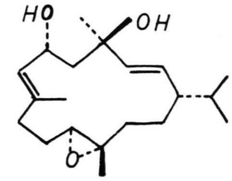

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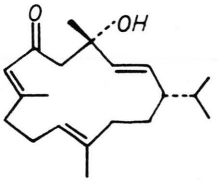

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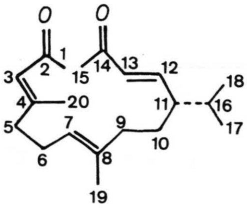

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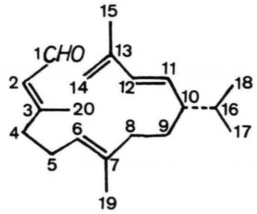

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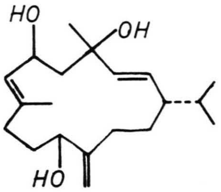

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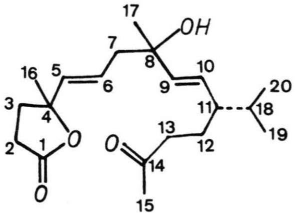

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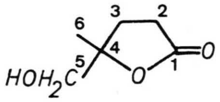

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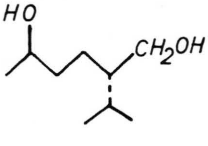

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structure of the triol (**13**) was established by its spectral data [13] (MS, ^{1}H-NMR, ^{13}C-NMR) to be 2,7,12 (**20**)-cembratriene-4,6,11-triol, its stereochemistry still remaining unknown. (1 S, 2 E, 4 S, 6 R, 7 E, 11 S)-2,7,12 (20)-cembratriene-4,6,11-triol is said to be found in Turkish tobacco [14].

Furthermore a new natural product was isolated, suggesting the structure of the *seco*-lactone (**14**).

Precise mass determination was accomplished by FD-mass spectra yielding the molecular weight 336, confirming the elemental composition $C_{20}H_{32}O_4$ [15]. The ^{1}H-NMR-spectrum implied the presence of an

Table I. ^{13}C-NMR chemical shifts and assignments for compounds $\mathbf{5}_{1-3}$, **10**–**15**.

	C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
$\mathbf{5}_1$[d]	47.8	128.6	139.6	72.4[c]	48.4	128.4	141.9	71.9[c]	44.1	27.9
$\mathbf{5}_2$[d]	47.9	128.3	140.2	72.3[c]	48.9	127.4	142.0	72.6[c]	43.8	28.3
$\mathbf{5}_3$[d]	47.8	128.8	140.7	72.6[c]	49.1	126.6	141.7	72.7[c]	43.0	28.4
10[e]	46.9	126.6	136.2	72.5	54.2	201.9	131.1	159.9	31.8[b]	28.9[c]
11[e]	31.6	198.5[b]	132.2	157.8	41.0	25.0	123.3	135.6	37.4	29.7
12[e]	190.9	127.4	163.4	40.7	25.7	122.3	136.9	37.7	30.7	49.1
13[f]	49.0	130.2	138.6	72.5	53.7	65.2	131.2	138.8	33.4[b]	31.9[b]
14[e]	176.4	28.9	34.2	85.1	135.9	125.3	45.6	72.2	138.4	129.8
15[e]	177.0	29.5[a]	29.6[a]	86.4	23.1	68.5				

	C-11	C-12	C-13	C-14	C-15	C-16	C-17	C-18	C-19	C-20
$\mathbf{5}_1$	123.3	131.5	37.8	23.1	33.6	20.4[a]	19.9[a]	27.9[b]	27.8[b]	14.3
$\mathbf{5}_2$	123.1	131.5	37.8	23.2	33.7	20.8[a]	19.9[a]	29.9[b]	28.4[b]	14.3
$\mathbf{5}_3$	122.2	131.0	37.7	22.4	33.5	20.9[a]	20.0[a]	30.6[b]	29.6[b]	14.1
10	123.5	134.6	36.3[b]	25.7[c]	32.2	20.1[a]	19.9[a]	30.0	24.8	14.9
11	48.8	150.4	123.6	198.0[b]	26.9	31.6	20.5[a]	19.1[a]	15.9	26.5
12	133.9	132.7	142.1	114.0	18.8	32.1	20.7[a]	19.0[a]	16.1	17.6
13	73.6	153.5	36.0[b]	31.6[b]	33.0	21.1[a]	19.0[a]	31.5	16.8	110.6
14	48.7	26.3	42.0	209.3	29.9	26.6[b]	27.9[b]	32.0	20.6[a]	19.1[a]

[a] may be reserved; [b] may be reserved; [c] may be reserved; [d] δ-values in $C_4D_8O_2$; [e] δ-values in $CDCl_3$; [f] δ-values in CD_3OD.

isopropyl group ($\delta = 0.84$ ppm, 3H, d, $J = 6.7$ Hz, $\delta = 0.89$ ppm, 3H, d, $J = 6.7$ Hz), and three methyl groups ($\delta = 1.29$ ppm, 3H, S, $\delta = 1.52$ ppm, 3H, s, $\delta = 2.16$ ppm, 3H, s), one of them evidently attached to a carbonylfunction ($\delta = 2{,}16$ ppm) and four olefinic protons. This was consistent with the ^{13}C-NMR properties, which revealed one carbonylfunction ($\delta = 209.3$ ppm), one carboxylfunction ($\delta = 176.4$ ppm) and two further oxygene bearing carbons at $\delta = 72.7$ ppm (s) and $\delta = 85.1$ ppm (s). The proposed structure was ensured by ozonolysis of (**14**), followed by reduction with $NaBH_4$, yielding the diol (**16**) and the hydroxylactone (**15**). The spectral properties of the diol (**16**) proved to be in accordance with those of an authentic sample and literature data [16]. The hydroxylactone (**15**), prepared by Grignard reaction of ethyllevulinate with 1-bromopropene followed by ozonolysis and reduction with $NaBH_4$, showed the same spectral data as the product, obtained by ozonolyses of (**14**) [17].

This investigation establishs the complexity of the distribution of diterpenes and supports the degradation schemes recently reported [14]. The identification of the *seco*-lactone (**14**) gives for the first time a strong hint of a similar oxidative cleavage of 11,12-double bond in 4,8-diols (**5**) as reported for 4,6-diols [14].

Acknowledgement

Thanks are due to Dr. F. Borchert, Kali-Chemie AG, for the FD-MS.

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[13] ^{1}H-NMR (CD_3OD), Bruker WH 270: 0.84 (6H, t, $J =$ 6.7 Hz), 1.29 (3H, s), 1.72 (3H, d, $J = 1.3$ Hz), 3.97 (1H, t, $J = 6.5$ Hz), 4.64 (1H, dt, $J = 2.1$ and 9 Hz), 4.86 (1H, s), 5.02 (1H, s) 5.20 (1H, d, $J = 9$ Hz), 5.33 (1H, dd, $J = 15.5$ Hz, $J = 6.9$ Hz), 5.43 (1H, d, $J =$ 15.5 Hz). MS *m/e* %: 304 (2%); 286 (9%); 268 (6%); 243 (8%); 225 (9%); 159 (17%); 145 (33%); 133 (35%); 119 (41%); 79 (70%); 69 (55%); 55 (75%); 43 (100%); 41 (98%).

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[15] Found ($M^{\cdot +} - H_2O$): 318.2188; Calc. for $C_{20}H_{30}O_3$ ($M^{\cdot +} - H_2O$): 318.2195. ^{1}H-NMR: 0.84 (3H, d, $J =$ 6.7 Hz), 0.89 (3H, d, $J = 6.7$ Hz), 1.29 (3H, s), 1.52 (3H, s), 216 (3H, s) 5,33 (1H, dd, $J = 15.6$ Hz, $J = 9$ Hz), 5.48 (1H, d, $J = 15.6$ Hz), 5.63 (1H, d, $J = 15.6$ Hz), 5.76 (1H, m). MS *m/e* (%): 318 (8%), 300 (1%), 285 (4%), 260 (2%), 217 (4%), 197 (30)%, 179 (16%), 161 (26%), 140 (87%), 121 (51%), 95 (58%), 71 (38%), 55 (26%), 43 (100%).

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[17] ^{1}H-NMR: 1.33 (3H, s), 1.88 (1H, ddd, $J = 10.0$ Hz, $J = 6.8$ Hz, $J = 12.8$ Hz), 2.33 (1H, ddd, $J = 10$ Hz, $J =$ 6.8 Hz, $J = 12.8$ Hz), 2.57 (1H, ddd, $J = 17.8$ Hz, $J =$ 10 Hz, $J = 5.8$ Hz) 2.67 (1H, ddd, $J = 17.8$ Hz, $J = 10$ Hz, $J = 6{,}8$ Hz), 3.48 (1H, d, $J = 12.2$ Hz), 3.67 (1H, d, $H =$ 12.2 Hz). MS *m/e* (%): 115 (3%), 112 (1%), 99 (100%), 71 (13%), 56 (11%), 43 (85%).