A New Cembranoid from Tobacco, IV *

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Z. Naturforsch. **39 c,** 1023–1026 (1984); received May 30, 1984

Dedicated to Austria Tabak Werke AG, vormals österreichische Tabakregie, on the Occasion of Their 200th Anniversary

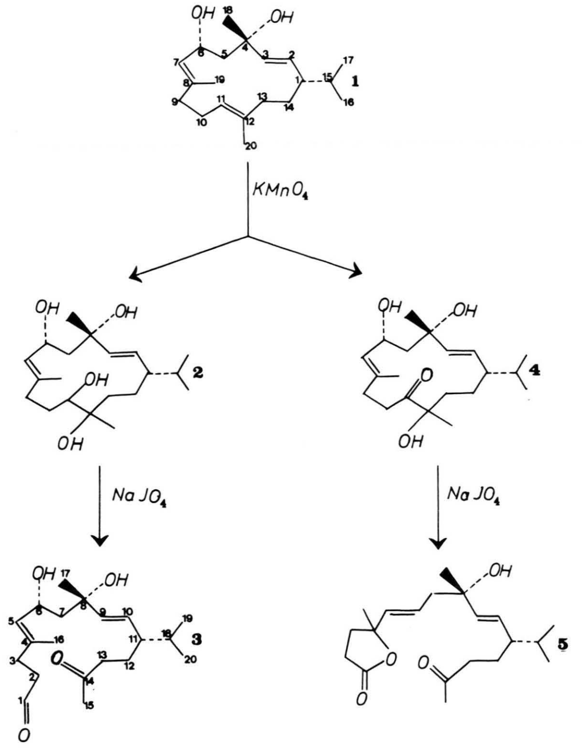
Nicotiana tabacum, Solanaceae, Diterpenes, Cembranoids, seco-Ketolacton, Degradation

4,8-Dimethyl-11-isopropyl-6,8-dihydroxy-pentadeca-4,9-dien-14-on-1-al (**3**) was identified as a new natural product from tobacco. **3** was isolated from the surface gum of fresh tobacco. The spectral data, chemical properties and the synthesis **3** 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 [2, 3]. Bioconversion and biodegradation of these compounds leads to a large number of diterpenoid-derivatives and norterpenoids which are important tobacco flavour substances [4].

The methanol extracts of the leaf surface gums from burley and virginia tobacco were fractionated on silica gel and the crude fractions were subjected to repeated column chromatography, TLC, and HPLC. In addition to the 2,7,11-cembratriene-4,6-diols (**1**) the new tobacco constituent **3** was isolated and identified by MS [5], 13-C-NMR [6], 1-H-NMR [7], 2-D–1H–1H shift-correlation spectroscopy [8] and 2-D–1H–13C shift-correlation spectroscopy [8].

The 1H-NMR spectrum reveals a very complex pattern of overlapping signals and even double resonance experiments gave no significant information. Signals were assigned by 2-D–1H–1H shift correlation spectroscopy (Jeener-type). Starting at the signal for 1-H (9.75 ppm) the signals for the two methylen-groups at 2-C (2.56 ppm) and 3-C (2.13 ppm) are easily detected by their cross-peaks.



The doublett of 5-H (5.22 ppm) shows cross-peaks with 6-H (4.77 ppm) and 16-CH3 (1.69 ppm), while cross-peaks appear also between 6-H, 7-H (1.74 ppm) and 7″-H (1.47 ppm). By evaluating cross-peaks the next partial structural element of **3** starts at the doublett for 9-H (5.48 ppm) and the signals for 10-H (5.32 ppm), 11-H (1.47 ppm) the two protons

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* Part III see [1].

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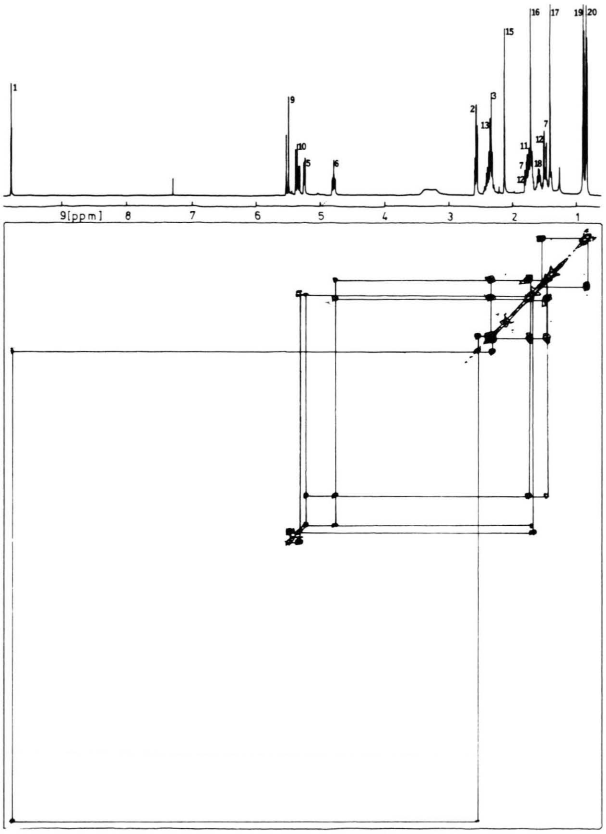


Fig. 1. Contour plot of the 1H−1H shift correlation Jeener-spectrum of **3** (solvent CDC13, 400.13 MHz).

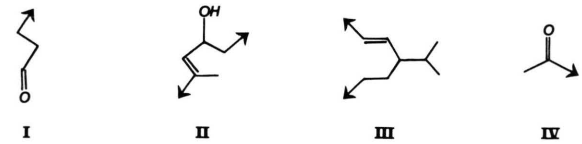


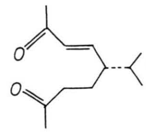
Fig. 2. Partial structures **I–IV**.

12-H (1.73 ppm), 12″-H (1.47 ppm) and 13-H (2.73 ppm) are assigned. Cross-peaks with 11-H reveals 18-H (1.57 ppm) and the two methyl-groups 19-CH3 and 20-CH3 (0.87 ppm and 0.82 ppm). The shift of 15-CH3 (2.11 ppm) indicates an acetyl-group. By evaluation of the foregoing 2-D−1H−1H shift correlation spectrum of **3** (Fig. 1) the partial structures **I, II, III** and **IV** are determined (Fig. 2).

Since the Jeener-spectrum established the assignment of protons and their connections the identification of carbons was done by means of 2-D−1H−13C shift correlation spectroscopy (Fig. 3). Determination of 4-C (135.48 ppm), 8-C (73.02 ppm), and 14-C (208.95 ppm) is not possible since they bear no directly attached proton, but the chemical shift indicates partial structures **V** and **VI** (Fig. 4).

The connection of fragments **I–IV** is established by comparison with NMR-data of **1, 6** and **7,** by chemical properties (self-degradation) of **3,** and its synthesis.

The seco-Ketoaldehyde **3** is an important tobacco constituent in terms of degradation of tobacco cembranoids because it undergoes selfdegradation, especially to the very well known tobacco nor-diterpenoids solanone (**6**) and norsolandione (**7**). Analytical data of several freshly prepared tobacco gums exhibit a strong relation between the concentration of **3** and the concentration of solanone (**6**) and norsolandione (**7**): if the concentration of the seco-ketoaldehyde **3** is high, the concentration of the nor-diterpenoids is low and vice versa. This explains an old observation that aging of fractions of polar diterpenes causes a growing amount of norditerpenes.



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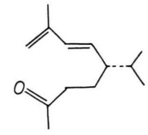

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In order to prove our structure elucidation of **3** we undertook a two step synthesis [9] starting with (1*S*,2*E*,4*S*,6*R*,7*E*,11*E*)-cembratiene-4,6-diol (**1**). This cembranoid, occurring in large amounts in tobacco, was oxidized with $KMnO_4$ in either slightly acidic medium to yield the acyloin (**4**) [10] or in a slightly alkaline medium to yield the tetraol (**2**) accompanied by small amounts of compound **4**; both substances were purified by preparative HPLC [11]. Oxidation of the tetraol with $NaIO_4$ in dioxane/water gave **3** in nearly quantitative yield. Comparison of the spectral data of this synthesized material with the product isolated from tobacco gum showed that both were identical. Applying this glycolic cleavage to **4** resulted in formation of 4,8-dimethyl-8-hydroxy-11-isopropyl-14-oxo-pentadeca-5,9-diene-4-olide (**5**) [12] in 83% yield after two weeks reaction time. Obviously, this must be a two step reaction, since during the course of the reaction a very polar spot appears in the TLC; this spot might be 6,8-dihydroxy-11-isopropyl-4,8-dimethyl-14-oxo-4,9-pentadecadienoic acid [13],

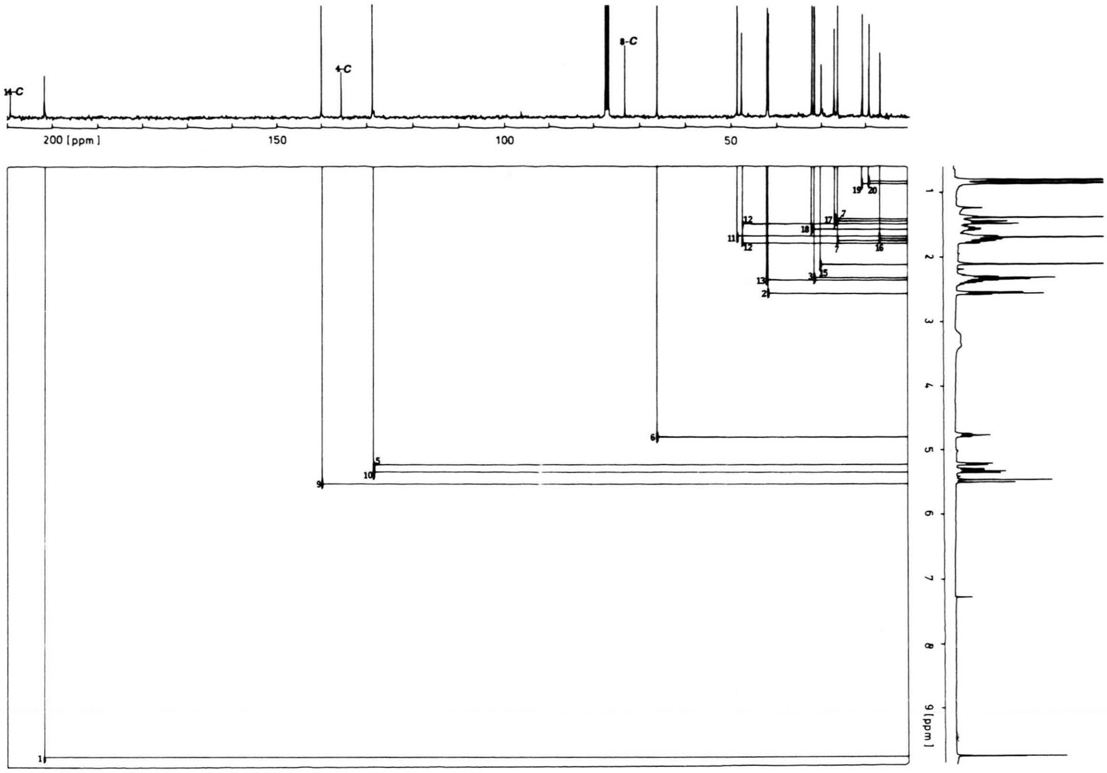

Fig. 3. Contour plot of the 1H−13C shift correlation spectrum of **3** (solvent CDC13, 100.62 MHz).

V **VI**

Fig. 4. Structural elements **V** and **VI**.

which undergoes an allylic rearrangement. In our hands we were not able to isolate this compound in the reaction mixture even working in pyridine solution.

These present results provide support for the view that the oxidation and degradation of the 4,6-diols might be initiated by enzyme catalyzed oxidations at the 11,12-double bond to form the hydroperoxides recently identified in tobacco [14], which are possibly cleaved by a hydroperoxide cleavage enzyme to form **3**. The seco-ketoaldehyde undergoes i.a. a retro-Prins-reaction to form solanone (**6**) and norsolandione (**7**).

Acknowledgement

We thank Dr. Robin A. Crellin GR & DC, British-American Tobacco Co. Ltd., Southampton, for assistance with the manuscript.

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[5] MS (70 eV) *m/z* (%): 320 ($M^{+\cdot} - H_2O$, 0.2), 302 ($M^{+\cdot} - 2H_2O$, 3.6), 136 (22), 121 (28), 93 (87), 43 (100).

[6] 13-C chemical shifts (ppm) in CDC13: 1-C (201.48), 2-C (41.77), 3-C (31.47), 4-C (135.48), 5-C (128.75), 6-C (66.14), 7-C (26.23), 8-C (73.02), 9-C (139.96), 10-C (128.91), 11-C (48.63), 12-C (47.83), 13-C

(41.96), 14-C (208.95), 15-C (29.77), 16-C (16.55), 17-C (26.23), 18-C (31.96), 19-C and 20-C (20.52 and 19.04). Signals for 5-C and 10-C may be reversed.

[7] 1-H chemical shifts (ppm) in CDCl3: 1-H (9.75), 2-H and 2″-H (2.56), 3-H and 3″-H (2.31), 5-H (5.22), 6-H (4.77), 7-H (1.74), 7″-H (1.47), 9-H (5.48), 10-H (5.32), 11-H (1.71), 12-H (1.73), 13-H and 13″-H (2.37), 18-H (1.57), 15-CH3 (2.11), 16-CH3 (1.69), 17-CH3 (1.39), 19-CH3 and 20-CH3 (0.87 and 0.82). Coupling constants (Hz): *J*1, 2 (1.7), *J*5, 6 (8.0), *J*5, 16 (1.3), *J*6, 7 (2.4), *J*6, 7″ (10.4), *J*9, 10 (15.6), *J*10, 11 (9.2).

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