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APPLICATION OF 2D-NMR SPECTROSCOPY IN THE STRUCTURAL DETERMINATION OF A NEW TOBACCO CEMBRANOID[1]

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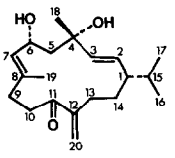
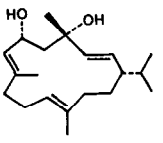
Abstract. Proton-proton shift correlated 2D-NMR spectroscopy has been used to determine the structure of a new tobacco cembranoid as (1S,2E,4S,6R,7E)-4,6-dihydroxy-2,7,12(20)-cembratrien-11-one (1). This assignment has been verified by chemical means.

Recent studies have shown that the cuticular wax of the leaf and flower of most tobacco varieties contains a wide array of diterpenoids of the cembrane class, the (1S,2E,4S,6R,7E,11E)- and (1S,2E,4R,6R,7E,11E)-2,7,11-cembratriene-4,6-diols (2, 3) being the major components and present in a substantial amount. These two diols (2, 3) have been postulated to give rise to the majority of the other tobacco cembranoids by biotransformations such as oxidations, acid-induced rearrangements and dehydrations. We now report the isolation and structure determination, using 2D-NMR spectroscopy, of a new tobacco cembranoid (1), which is a plausible metabolite derived from the 4S,6R-diol 2.[2]



1



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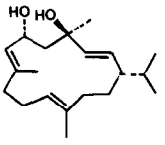


3

The new compound (1), $C_{20}H_{32}O_3$,[3] was obtained in a 2.1 mg yield from fraction C (6.2 g) of an extract (83 g) derived from flowers of Greek tobacco[4] by HPLC using a column packed with PrepPak-500/C_{18} followed by flash chromatography over silica gel and HPLC on Spherisorb 5 Nitrile and Spherisorb 5 columns. An analysis of its spectral data showed that 1 contains an oxo group conjugated with an exocyclic methylene group [IR bands at 1665 and 1625 cm^{-1}; signals at δ 5.69 and 6.00 in the ^1H NMR spectrum ($CDCl_3$)]. The remaining two oxygen atoms are accommodated by a secondary and a tertiary hydroxyl group

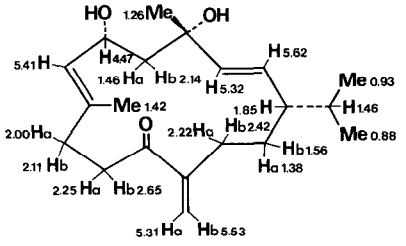


Table 1

$J_{1,2}$	=	9.7 Hz
$J_{1,14a}$	=	9.6 Hz
$J_{1,14b}$	=	4.0 Hz
$J_{1,15}$	=	6.7 Hz
$J_{2,3}$	=	15.9 Hz
$J_{5a,5b}$	=	-14.4 Hz
$J_{5a,6}$	=	1.3 Hz
$J_{5b,6}$	=	7.1 Hz
$J_{6,7}$	=	9.3 Hz
$J_{7,19}$	=	1.3 Hz
$J_{9a,9b}$	=	-14.4 Hz
$J_{9a,10a}$	=	10.0 Hz
$J_{9a,10b}$	=	4.1 Hz
$J_{9b,10a}$	=	4.2 Hz
$J_{9b,10b}$	=	7.1 Hz
$J_{10a,10b}$	=	-14.6 Hz
$J_{13a,13b}$	=	-17.2 Hz
$J_{13a,14a}$	=	6.6 Hz
$J_{13a,14b}$	=	10.5 Hz
$J_{13b,14a}$	=	10.5 Hz
$J_{13b,14b}$	=	3.9 Hz
$J_{14a,14b}$	=	-14.0 Hz
$J_{15,16}$	=	6.7 Hz
$J_{15,17}$	=	6.7 Hz
$J_{20a,20b}$	=	1.7 Hz

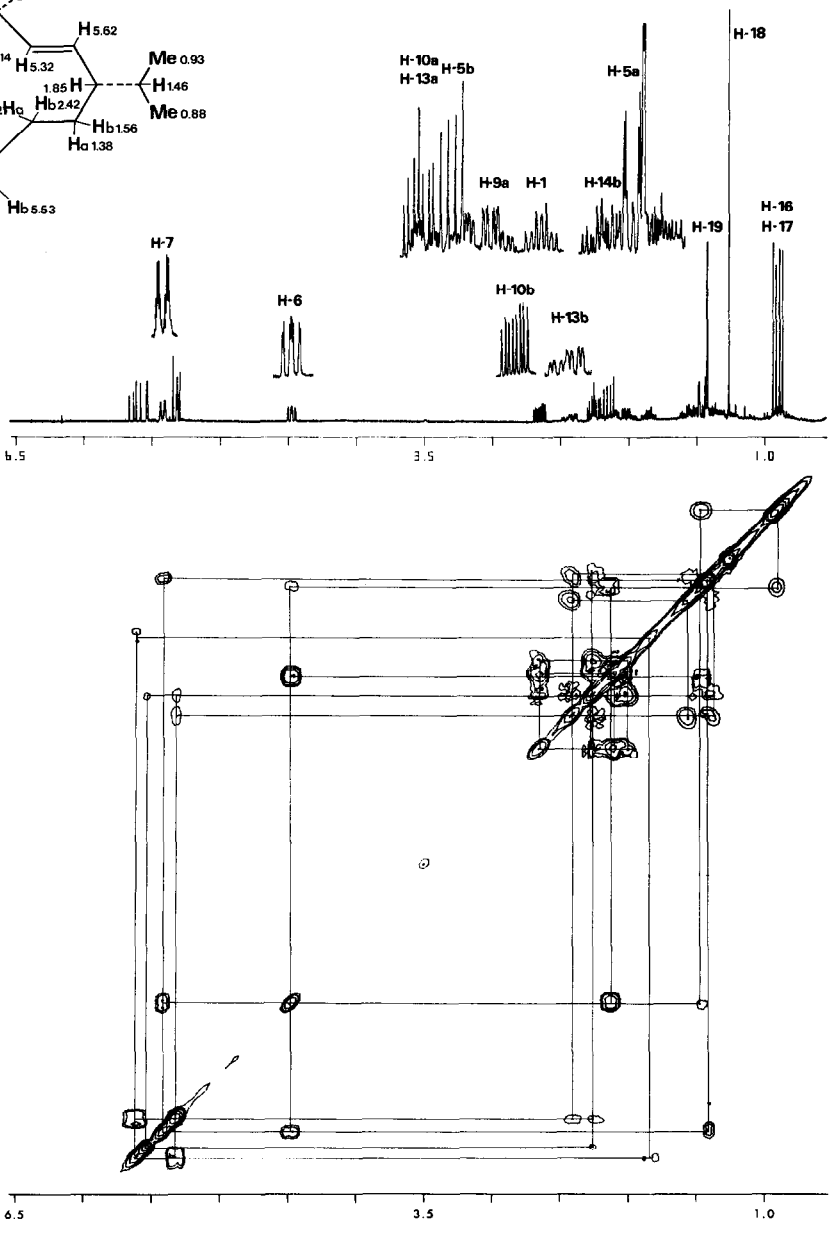


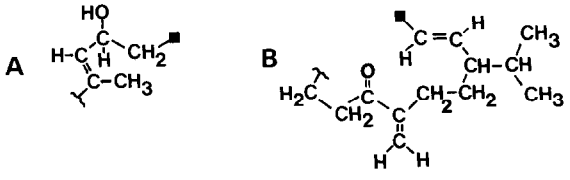
Fig. 1a. 300 MHz ^{1}H NMR spectrum of 1 run in C_6D_6.

Fig. 1b. Contour plot of the ^{1}H - ^{1}H shift correlation (Jeener) spectrum of 1 run in C_6D_6. The map is composed of 512 x 512 data point spectra, each consisting of 96 transients. The evolution period, t_1, was incremented in 256 steps. The mixing pulse was reduced to 45° and a pseudo-echo "shaping function" [7] was used in both time dimensions.

[OH-absorption in the IR spectrum; ^{13}C NMR signals at δ 67.9 (d) and 73.5 (s)]. These results and the presence of two additional double bonds, of which one is disubstituted and one is trisubstituted, demonstrated that 1 is carbomonocyclic.

The occurrence of an isopropyl group (methyl doublets at δ 0.85 and 0.92 ; IR bands at 1384 and 1369 cm^{-1}) and two methyl groups, of which one is attached to the carbon atom carrying the tertiary hydroxyl group and the other being vinylic (methyl signals at δ 1.21 and 1.69), reinforced our view that 1 is a diterpenoid of the cembrane class.

Proton-proton shift correlated 2D-NMR spectroscopy was used to confirm this assignment.[5,6] Thus, the Jeener-spectrum shown in Fig. 1 (C_6D_6) delineated the correlation of almost all protons in 1; the couplings between H-1 and H-15, between H-1 and the two protons at C-14 and between the two protons at C-20, which were not ascertained due to low intensities of the cross peaks, being revealed by the presence of appropriate cross peaks in the $CDCl_3$ spectrum. With the aid of these results an analysis of the 1D-spectrum was undertaken. The coupling (Table 1) and chemical shift information thus obtained allowed the formulation of structural fragments A and B. Since the 1,2-disubstituted and trisubstituted double bonds are not conjugated, these can only be combined via the carbon atom carrying the tertiary hydroxyl group to form a 4,6-dihydroxy-2,7,12(20)-cembratrien-11-one structure.

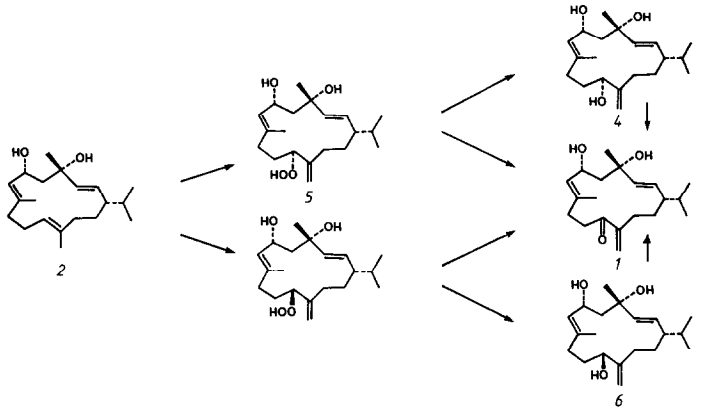


The present study, in which the chemical shifts and coupling constants of all protons in a tobacco cembranoid have been determined for the first time, illustrates the advantage of homoscalar-correlated 2D-NMR spectroscopy over traditional decoupling experiments. This is particularly true for cases like 1 where coupled protons have small chemical shift differences, e. g. those resonating in the methylene region of the spectrum.

With the gross structure of 1 at hand, a comparison of the ^{13}C NMR spectra of 1 and (1S,2E,4S,6R,7E,11S)-2,7,12(20)-cembratriene-4,6,11-triol (4)[8] proved fruitful. Thus, since the chemical shifts of the signals assigned to C-2 to C-8 and C-18 for 1 were close to those of the corresponding signals for 4, it was concluded that 1 has a 2E,4S,6R,7E-stereochemistry.

This assignment was readily verified by treatment of (1S,2E,4S,6R,7E,11S)-11-hydroperoxy-2,7,12(20)-cembratriene-4,6-diol (5)[9] with $CuCl_2$ in chloroform.[10] The major product, (1S,2E,4S,6R,7E)-4,6-dihydroxy-2,7,12(20)-cembratrien-11-one, which is formed by dehydration, proved to be identical to the new tobacco isolate (1).

This route to 1 may resemble that occurring in tobacco, a conclusion supported by the fact that the 11S-hydroperoxide 5, which is a plausible metabolite of the 4S,6R-diol 2, has recently been isolated from tobacco (Scheme 1). An alternative route to 1 would involve regioselective oxidation of the 4S,6R,11S- and/or 4S,6R,11R-triol (4, 6) both of which are tobacco constituents.[4,8]



Scheme 1.

Acknowledgements. We are grateful to Dr. R. Freeman, Oxford University, and to Dr. G.A. Morris, University of Manchester, for gifts of software and for valuable advice.

References and notes

1. Part 61 in the series Tobacco Chemistry.
2 I. Wahlberg and C.R. Enzell, Beiträge Tabakforsch., in press.
3. 1 was a gum, which had $[\alpha]_D$ -16° (c 0.15, $CHCl_3$); IR ($CHCl_3$) bands at 3602, 3480, 3090, 1665, 1625, 1384 and 1369 cm^{-1}; MS [m/z (%, composition)]: 320 (M, 0.2), 302 (2, $C_{20}H_{30}O_2$), 287 (1, $C_{19}H_{27}O_2$), 284 (0.8, $C_{20}H_{28}O$), 277 (1, $C_{17}H_{25}O_3$), 259 (4), 241 (2), 231 (3, $C_{16}H_{23}O$), 206 (4, $C_{14}H_{22}O$), 137 (13), 121 (18), 109 (20, C_8H_{13} and C_7H_9O), 95 (31, C_7H_{11} and C_6H_7O), 81 (28, C_6H_9), 69 (29, C_5H_9 and C_4H_5O), 55 (33, C_4H_7 and C_3H_3O) and 43 (100, C_2H_3O and C_3H_7); ^1H NMR ($CDCl_3$): δ 0.85 (d, J = 6.8 Hz)/0.92 (d, J = 6.8 Hz) (H-16/H-17), 1.21 (s, H-18), 1.65 (dd, J = 1.3 and -14.6 Hz, H-5a), 1.69 (d, J = 1.5 Hz, H-19), 2.18 (dd, J = 7.1 and -14.6 Hz, H-5b), 4.66 (ddd, J = 1.3, 7.1 and 9.4 Hz, H-6), 5.50 (dq, J = 1.5 and 9.4 Hz, H-7), 5.4-5.6 (overlapping signals, H-2 and H-3), 5.69 (t, J = 1.6 Hz, H-20a) and 6.00 (d, J = 0.9 Hz, H-20b); ^{13}C NMR ($CDCl_3$): δ 46.0 (C-1), 128.4 (C-2), 139.1 (C-3), 73.5 (C-4), 45.3 (C-5), 67.9 (C-6), 128.4 (C-7), 137.7 (C-8), 28.5 (C-9), 36.8 (C-10), 203.4 (C-11), 147.3 (C-12), 35.0 (C-13), 27.0 (C-14), 32.4 (C-15), 19.5/20.9 (C-16/C-17), 31.4 (C-18), 16.0 (C-19) and 122.3 (C-20).
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7. A. Bax, R. Freeman and G.A. Morris, J. Magn. Reson. 43 (1981) 333.
8. I. Wahlberg, I. Wallin, C. Narbonne, T. Nishida, C.R. Enzell and J.-E. Berg, Acta Chem. Scand., B36 (1982) 147.
9. I. Wahlberg, K. Nordfors, C. Vogt, T. Nishida and C.R. Enzell, Acta Chem. Scand., B37 (1983) 653.
10. To 10 mg of 5 in 3 ml of chloroform was added 5 mg of $CuCl_2 \times 2H_2O$. The reaction mixture was stirred at room temperature for 24 h, washed with water, dried and concentrated. The residue was separated by HPLC (Spherisorb 5 Nitrile, hexane/ethyl acetate 40:60) to afford 1.2 mg of 1.

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