Acta Chemica Scandinavica B 33 (1979) 541—543

Tobacco Chemistry. 49.* New Labdanic Diterpenoids Isolated from Tobacco

INGER WAHLBERG,[a] INGRID WALLIN,[a] KERSTIN NORDFORS,[a] TOSHIAKI NISHIDA,[a] CURT R. ENZELL [a] and WILLIAM W. REID [b]

[a] Research Department, Swedish Tobacco Company, P.O. Box 17007, S-104 62 Stockholm, Sweden and [b] Chemistry Department, Queen Elizabeth College, University of London, Campden Hill, London, Great Britain

Seventeen labdanic compounds have been isolated from *Nicotiana tabacum* PB (Bergerac). Of these, eight are new tobacco constituents and seven, the (12*S*, 13*S*)-, (12*R*, 13*R*)- and (12*R*, 13*S*)-8,13-epoxy-14-labden-12-ols (*9*, *10*, *12*), 12,15-epoxy-12,14-labdadien-8-ol (*13*), the (11*E*, 13*S*)- and (11*E*, 13*R*)-11,14-labdadiene-8,13-diols (*14*, *15*) and (13*E*)-15-acetoxy-13-labden-8-ol (*17*) are new natural products. The isolation of compounds *9*, *10*, and *12-15*, all of which have previously been obtained as products of the reaction of (12*Z*)- and/or (12*E*)-abienol (*1*, *2*) with singlet oxygen, strongly corroborates current views on the biogenesis of the tobacco labdanoids.

Recent studies have shown that depending on their genetic origin tobacco cultivars produce cembranes, labdanes or both. These diterpenoids, which are present in substantial amounts in the cuticular wax of the tobacco leaf and hence susceptible to light and oxidation, are likely to give rise to many of the odoriferous nor-compounds present in processed tobacco.[2–4]

Although only the (12*Z*)-isomer had so far been encountered,[2] it was hypothesized [4,5] and subsequently inferred from model experiments involving sensitized photo-oxygenation [1,6] that both (12*Z*)- and (12*E*)-abienol (*1*, *2*) are precursors of the majority of the tobacco labdanoids and nor-labdanoids. However, since some of the key photo-oxygenation products were not known as tobacco constituents, a search for these was deemed to be of interest.

To this end, *Nicotiana tabacum* PB (Bergerac), the principal commercial variety cultivated in France and known to contain (12*Z*)-abienol (*1*) and (13*E*)-13-labdene-8,15-diol (*3*),[2,3] was selected. The present report describes an examination of chloroform extracts of green, yellowed and dried (cured) leaves and fresh flowers from this species.

RESULTS

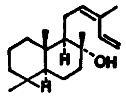
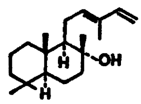
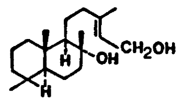
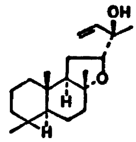
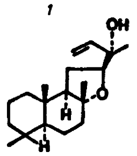
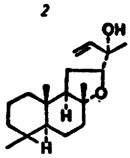
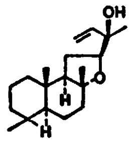
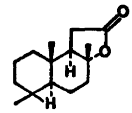
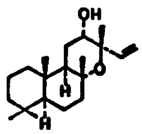
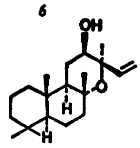
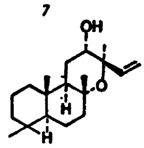
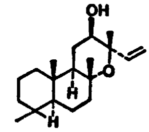
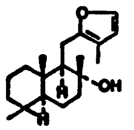
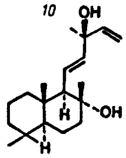
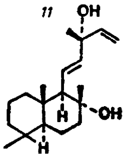
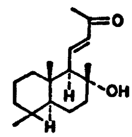
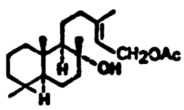
A comparative GC-MS study showed that the various extracts contained mainly paraffin hydrocarbons and labdanoids and had fairly similar compositions, the major difference being the concentration of (12*Z*)-abienol (*1*), which was considerably higher in green leaves and flowers than in yellowed and dried leaves.

Separation of the extracts of green leaves and flowers using HPLC resulted in the isolation not only of the two presumed precursors (12*Z*)- and (12*E*)-abienol (*1*, *2*), but also of the (12*R*,13*R*)-,[6] (12*S*,13*S*)-,[6] (12*R*,13*S*)-,[1] and (12*S*,13*R*)-8,12-epoxy-14-labden-13-ols[1] (*4-7*), norambreinolide (*8*),[1,6] the (12*S*,13*S*)-,[6] (12*R*,13*R*)-,[6] (12*S*,13*R*)-[1] and (12*R*,13*S*)-8,13-epoxy-14-labden-12-ols[1] (*9-12*), 12,15-epoxy-12,14-labdadien-8-ol (*13*),[1,6] the (11*E*,13*S*)- and (11*E*,13*R*)-11,14-labdadiene-8,13-diols (*14*, *15*)[1,6] and (11*E*)-14,15-bisnor-8-hydroxy-11-labden-13-one (*16*),[1,6] all of which have previously been obtained by photo-oxygenation of (12*Z*)- and/or (12*E*)-abienol (*1*, *2*).

Of these, compounds *2*, *9*, *10* and *12–15* are new to tobacco and compounds *9*, *10* and *12–15* are new natural products. Their isolation from tobacco strongly reinforces the view that

* For part 48: see Ref. 1.

0302-4369/79/070541-03$02.50


1

2

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17

the majority of the labdanoids and nor-labdanoids of tobacco are generated by oxidative transformations of (12*Z*)- and (12*E*)-abienol (*1*, *2*).

(13*E*)-13-Labdene-8,15-diol (*3*) and the corresponding monoacetate *17*, which are probably not formed *via* the abienols (*1*, *2*), were also isolated. Acetate *17* is a new tobacco constituent, and, to our knowledge, also a new natural product.

EXPERIMENTAL

For instrumental details, see Ref. 7.

Extraction. Green, yellowed and dried leaves and fresh flowers of *Nicotiana tabacum* PB (Bergerac) were separately packed into extractors (5 l) and percolated with $CHCl_3$. The resultant extracts were concentrated in a rotary evaporator and dried for 18 h at 45 °C *in vacuo.*

A GC-MS study showed that in addition to paraffin hydrocarbons, compounds *1*–*17*, whose isolation is described below, were present in all four extracts.

Isolation. Part of the extract (3 g) obtained from green leaves was chromatographed over silica gel to give ten fractions. Each of these was separated further by HPLC using columns packed with μ-Porasil, μ-Bondapak C_{18} or μ-Bondapak CN to give the (12*R*,13*R*)-, (12*S*,13*S*)-, (12*R*,13*S*)- and (12*S*,13*R*)-8,12-epoxy-14-labden-13-ols (*4*–*7*),[5,8,9] norambreinolide (*8*),[10] the (12*R*,13*R*)-,(12*S*,13*R*)- and (12*R*,13*S*)-8,13-epoxy-14-labden-12-ols (*10*–*12*),[5,9,11] the (11*E*,13*S*)- and (11*E*,13*R*)-11,14-labdadiene-8,13-diols (*14*,*15*),[6] (11*E*)-14,15-bisnor-8-hydroxy-11-labden-13-one (*16*)[12] and (13*E*)-15-acetoxy-13-labden-8-ol (*17*),[13] all of which were identified by comparison with authentic samples.

A complementary separation of part of the extract (6.8 g) of fresh flowers yielded the additional (12*Z*)- and (12*E*)-abienol (*1*, *2*),[14,15] (13*E*)-13-labdene-8,15-diol (*3*),[16] (12*S*,13*S*)-8,13-epoxy-14-labden-12-ol (*9*)[5] and 12,15-epoxy-12,14-labdadien-8-ol (*13*),[6] which due to separation difficulties and decomposition could not be isolated from the extract of green leaves.

Acknowledgements. One of us (W. W. Reid) thanks the British American Tobacco Company for generous financial assistance, and Dr J. Chouteau of the Institut Experimental du Tabac Bergerac for growing the plants used in this work and providing laboratory facilities.

REFERENCES

1. Wahlberg, I., Nordfors, K., Curvall, M., Nishida, T. and Enzell, C. R. *Acta Chem. Scand. B 33* (1979) 437.
2. Colledge, A., Reid, W. W. and Russell, R. *Chem. Ind. London* (1975) 570.
3. Reid, W. W. *Ann. Tabac S.E.I.T.A. 2* (1974) 145.
4. Enzell, C. R., Wahlberg, I. and Aasen, A. J. *Fortschr. Chem. Org. Naturst. 34* (1977) 1.
5. Wahlberg, I., Curvall, M. and Enzell, C. R. *Acta Chem. Scand. B 32* (1978) 310.
6. Wahlberg, I., Karlsson, K., Curvall, M., Nishida, T. and Enzell, C. R. *Acta Chem. Scand. B 32* (1978) 203.
7. Behr, D., Wahlberg, I., Nishida, T. and Enzell, C. R. *Acta Chem. Scand. B 31* (1977) 573.
8. Aasen, A. J., Hlubucek, J. R. and Enzell, C. R. *Acta Chem. Scand. B 29* (1975) 589.
9. Wahlberg, I., Karlsson, K., Nishida, T., Cheng, K.-P., Enzell, C. R., Berg, J.-E. and Pilotti, A.-M. *Acta Chem. Scand. B 31* (1977) 453.
10. Hinder, M. and Stoll, M. *Helv. Chim. Acta 36* (1953) 1995.
11. Giles, J. A., Schumacher, J. N., Mims, S. S. and Bernasek, E. *Tetrahedron 18* (1962) 169.
12. Hlubucek, J. R., Aasen, A. J., Almqvist, S.-O. and Enzell, C. R. *Acta Chem. Scand. B 28* (1974) 131.
13. McCreadie, T. and Overton, K. H. *J. Chem. Soc. C* (1971) 312.
14. Gray, P. S. and Mills, J. S. *J. Chem. Soc.* (1964) 5822.
15. Mills, J. S. *J. Chem. Soc. C* (1967) 2514.
16. Asselineau, C., Bory, S., Fétizon, M. and Laszlo, P. *Bull. Soc. Chim. Fr.* (1961) 1429.

Received May 8, 1979.