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Isolation of a New Tobacco Constituent, (3S, 5R, 6S, 9ξ)-3-Hydroxy-5, 6-epoxy-β-ionol, from Japanese Domestic SUIFU Tobacco†

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We have previously reported the isolation of nor-iso prenoids related to tobacco thunberganoids from the Japanese domestic SUIFU tobacco.[1] In this paper we wish to report the isolation of a new compound related to tobacco carotenoids.

Compound **A** (7 mg), a yellow oil, was isolated from Fraction-11 described in the previous paper[1] by repeated column chromatography using silicic acid and preparative gas chromatography. The infrared spectrum of compound **A** showed clearly a broad hydroxyl absorption at 3400 cm^{-1}, *gem*-dimethyl absorption at 1382, 1376 cm^{-1} and a *trans* double bond absorption at 980 cm^{-1}. The PMR spectrum showed *gem*-dimethyls (δ 1.00, 3H, s; 1.16, 3H, s), one CH_3–CHOR group (δ 1.32, 3H, d, J=7 Hz; 4.46, 1H, m), one CH_3–COR group (δ 1.22, 3H, s), one CHOR group (δ 3.97, 1H, m) and two olefinic protons (δ 5.99, 2H, m). By acetylation of compound **A**, two proton multiplets at δ 4.46 and 3.97 shifted downfield by *ca.* 1.1ppm, indicating the presence of two secondary hydroxyl groups in compound **A**.[2] The mass spectrum of compound **A** showed a fragment of the highest mass at *m/e* 208 and the precise mass determination established the elemental composition as $C_{13}H_{20}O_2$, which seemed to correspond to that obtained by a loss of water from the molecular formula $C_{13}H_{22}O_3$. These data lead to structure **4**, 3-hydroxy-5,6-epoxy-β-ionol, for compound **A**.

The gross structure was confirmed synthetically by epoxydation of 3-hydroxy-β-ionol, **1**.[3] The MS and IR spectra of synthetic 3-hydroxy-5,6-epoxy-β-ionol were identical with those of compound **A**. In the PMR spectrum of the synthetic material, the methyl signal which was assignable to the C–5 methyl linked to the epoxide was given at δ 1.22 and 1.24 and the both signals showed the same peak area, each equivalent to one-half of a methyl signal. This fact implied that the synthetic material was a mixture of *trans*-epoxide $\mathbf{4}_a$ and *cis*-epoxide $\mathbf{4}_b$ in the ratio of 1 : 1. The C–5 methyl of compound **A** was shown at δ 1.22 (3H, s) indicating that compound **A** was either $\mathbf{4}_a$ or $\mathbf{4}_b$. In another experiment, acetylated **1** (3-acetoxy-β-ionol acetate **2**) was epoxidized and saponified to give a mixture of $\mathbf{4}_a$ and $\mathbf{4}_b$. In the PMR spectrum of the synthetic mixture the signal of the C–5 methyl linked to the epoxide was shown at δ 1.22 as a minor (3H×1/4, s) and at δ 1.24 as a major (3H×3/4, s). The steric course of the epoxidation of 3-acetoxy-β-ionone has been reported to give a *trans*-epoxide as a minor product because of steric hindrance caused by a quasi-axial C–1 methyl group.[4] Thus, the minor peak at δ 1.22 was assignable to *trans*-epoxide $\mathbf{4}_a$ and the major at δ 1.24 to *cis*-epoxide $\mathbf{4}_b$. Therefore, the relative structure of compound **A** the C–5 methyl signal of which was given at δ 1.22 (3H, s) was determined as *trans* 3-hydroxy-5,6-epoxy-β-ionol $\mathbf{4}_a$. The absolute configuration at the C–3, –5 and –6 of compound **A** was established as (3S, 5R, 6S)-configuration on the basis of the facts that the CD data of 3-acetoxy-5,6-epoxy-β-ionone, **6**, derived from compound **A** by DDQ oxidation and acetylation was of the same sign (a negative Cotton effect) as those published by Mori[4] and Enzell *et al.*[5] The absolute configuration at C–9 could not be determined owing to the small amount of sample.

A large number of nor-compounds derived from carotenoid precursors have been reported as aroma constituents of tobacco.[6,7] Since some carotenoids with epoxy rings, *e.g.* violaxanthin and neoxanthin, which have been shown to be abundant in green leaves of tobacco,[8] possess the same (3S, 5R, 6S)-configuration as isolated compound **A**, it is highly probable that under the influence of oxygen and light, or enzyme[6] oxidative cleavages of the C(9)–C(10) polyene side-chain of these carotenoids lead the formation of compound **A**.

EXPERIMENTAL

Method. All the spectra used in this study were recorded with the following instruments. IR spectrometer: Jasco IR-G; mass spectrometer for precise mass determination: Hitachi RMU-7 combined with a HITAC 10 II datalizer; PMR spectrometer: JNM-PS-100; rotation spectrometer: Yanaco OR-50; CD spectrometer: JASCO J-20.

Isolation. The procedures for the extraction of 150 kg Japanese air-cured tobacco, *Nicotiana tabacum* cv. SUIFU, and the fractionation of the extract were described in the previous communication.[1] Compound **A** (7 mg) was isolated from Fraction 11 using repeated silicic acid column and preparative gas chromatography. Preparative gas chromatography was carried out under the following conditions with a

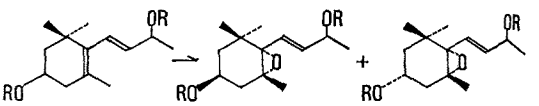
† Japanese Domestic Tobacco Flavor. Part II.[1]

Hitachi-063 gas chromatograph: column, 10% FFAP on chromosorb W, 3 mm×2 m; temperature, 150~250°C (5°C/min); carrier gas, He 50 ml/min.

Compound A, *(3S, 5R, 6S, 9ξ)-3-hydroxy-5,6-epoxy β-ionol.* MS (70 eV): *m/e* 208 (M^+–18, 26), 43 (100), 125 (53), 82 (25), 41 (21), 107 (17), 79 (17), 55 (17), 181 (15), 109 (15), 57 (15), 125 (13), 93 (13), 81 (13), 123 (11), 95 (11), 70 (11). Precise mass determination: $C_{13}H_{20}O_2$, found 208.1476, calcd. 208.1464. IR ν_{max}^{film}: 3380 (s), 2970 (s), 1660 (m), 1450 (m), 1382 (m), 1367 (s), 1147 (m), 1057 (s), 980 (s), 907 (m). $[\alpha]^{23}$: −77.3° (589), −68.4° (577), −76.4° (546), −151.1° (435), −253.8° (365), (*c*=0.2 in MeOH). PMR $\delta_{Me_4Si}^{CDCl_3}$: 1.00 (3H, s), 1.16 (3H, s), 1.22 (3H, s), 1.32 (3H, d, *J*=7 Hz), 3.97 (1H, m), 4.46 (1H, m), 5.99 (2H, m). Irradiation at δ 4.46 simplified a doublet at δ 1.32 to a singlet. Carbinol methine protons at δ 3.97 and 4.46 shifted to δ 5.04 and 5.50 by acetylation of compound A.

3-Hydroxy-5,6-epoxy-β-ionol **4** *(mixture of* $\mathbf{4_a}$ *and* $\mathbf{4_b}$*) from 3-hydroxy-β-ionol* **1**. A solution of *m*-chloroperbenzoic acid (20 mg) in $CHCl_3$ (1 ml) was added to an ice-cold solution of synthetic 3-hydroxy-β-ionol **1**[9] (crude 50 mg) in $CHCl_3$ (1 ml) at 0°C and the mixture was stirred overnight at 5°C. Then it was diluted with $CHCl_3$, washed with K_2CO_3 solution, dried (Na_2SO_4) and concentrated *in vacuo*. The residue showed only one main peak in preparative gas chromatography which was carried out under the same conditions as mentioned in the isolation of compound **A**, and the main peak gave 3-hydroxy-5,6-epoxy-β-ionol **4** (7 mg). The MS and IR spectra were identical with those of compound **A**. PMR $\delta_{Me_4Si}^{CDCl_3}$: 1.00 (3H, s), 1.16 (3H, s), 1.22 (3H×1/2, s), 1.24 (3H×1/2, s), 1.32 (3H, d, *J*=7 Hz), 3.97 (1H, m), 4.46 (1H, m), 5.99 (2H, m).

3-Hydroxy-5,6-epoxy-β-ionol **4** *from 3-acetoxy-β-ionol acetate* **2** *(acetylated* **1**). Synthetic 3-hydroxy-β-ionol **1** (crude 200 mg) was dissolved in dry pyridine (1 ml) and mixed with acetic anhydride (0.5 ml). The mixture was left standing overnight at room temperature, then 3-acetoxy-β-ionol acetate **2** (24 mg) was isolated by preparative gas chromatography from the reaction mixture. MS (70 eV): *m/e* 234 (M^+–18–42, 1), 159 (100), 43 (36), 131 (34), 91 (29), 174 (25), 41 (22), 144 (21), 177 (21), 105 (21). IR ν_{max}^{film}: 2975 (s), 1740 (s), 1455 (m), 1373 (s), 1243 (s), 1153 (m), 1142 (s), 973 (m), 952 (m), 610 (m). PMR $\delta_{Me_4Si}^{CDCl_3}$: 1.05 (3H, s), 1.19 (3H, s), 1.38 (3H, d, *J*=7 Hz), 1.71 (3H, s), 2.11 (6H, s), 5.15 (1H, m), 5.40~5.60 (2H, m), 6.16 (1H, d, *J*=16 Hz). A solution of *m*-chloroperbenzoic acid (30 mg) in $CHCl_3$ (1 ml) was added to an ice-cold solution of GC pure **2** (21 mg) in $CHCl_3$ (1 ml) at 0°C and the mixture was treated in the same manner as for the epoxidation of **1**→**4** to give 18 mg of crude 3-acetoxy-5,6-epoxy-β-ionol acetate **3** (mixture of *trans*-epoxide $\mathbf{3_a}$ and *cis*-epoxide $\mathbf{3_b}$). Crude **3** (15 mg) in MeOH (1 ml) was mixed with 1 N KOH (1 ml) and the mixture was left standing for 30 min at room temperature. It was diluted with water and extracted with ether. The ether layer was concentrated *in vacuo* and the residue gave 3-hydroxy-5,6-epoxy-β-ionol **4** (7 mg) by preparative gas chromatography under the above conditions. The MS and IR spectra were identical with those of compound **A**. PMR $\delta_{Me_4Si}^{CDCl_3}$: 1.00 (3H s), 1.16 (3H, s), 1.22 (3H×1/4, s), 1.24 (3H×3/4, s), 1.32 (3H, d, *J*=7 Hz), 3.97 (1H, m), 4.46 (1H, m), 5.99 (2H, m).



1, R=H **3**, R=Ac ($\mathbf{3_a}$) ($\mathbf{3_b}$)
2, R=Ac **4**, R=H Compound A ($\mathbf{4_a}$) ($\mathbf{4_b}$)

Epoxidation of **1** and **2**.

3-Acetoxy-5,6-epoxy-β-ionone $\mathbf{6}$[4,6] *from compound* A. A solution of 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ, 20 mg) in dry dioxane (1 ml) was added to a solution of compound **A** (4.5 mg) in dry dioxane (1 ml) at room temperature. After stirring overnight at 70°C, the product was chromatographed over alumina to give 3.1 mg of 3-hydroxy-5,6-epoxy-β-ionone **5**. MS (70 eV): *m/e* 224 (M^+, 1) 43 (100), 123 (82), 41 (32), 55 (12), 134 (11), 95 (11), 79 (11), 53 (11), 70 (10). IR ν_{max}^{film}: 3420 (s), 2960 (s), 1677 (s), 1628 (s), 1366 (s), 1260 (s), 1126 (m), 1043 (s), 983 (m), 918 (m). PMR $\delta_{Me_4Si}^{CDCl_2}$: 1.04 (3H, s), 1.22 (3H, s), 1.24 (3H, s), 2.34 (3H, s), 3.98 (1H, m), 6.36 (1H, d, *J*=16 Hz), 7.04 (1H, d, *J*=16 Hz). Acetic anhydride was added to a solution of **5** (3.1 mg) in the same manner as for the acetylation of **1**→**2**. Then 3-acetoxy-5,6-epoxy-β-ionone **6** (2.8 mg) was isolated by preparative gas chromatography. MS (70 eV): *m/e* 266 (M^+, 0), 251 (M^+–15, 1), 123 (100), 43 (90), 41 (12), 79 (6), 95 (5), 77 (5), 55 (5), 53 (5). IR ν_{max}^{film}: 2970 (m), 1738 (s), 1700 (m), 1680 (s), 1628 (m), 1365 (s), 1244 (s), 1032 (m), 982 (m). PMR $\delta_{Me_4Si}^{CDCl_3}$: 1.01 (3H, s), 1.21 (3H, s), 1.29 (3H, s), 2.06 (3H, s), 2.32 (3H, s), 5.10 (1H, m), 6.36 (1H, d, *J*=16 Hz), 7.02 (1H, d, *J*=16 Hz). CD: negative cotton effect (*c*=0.0060 in MeOH), $[\theta]_{231}$ −11200.

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REFERENCES

1) Y. Takagi, T. Chuman, T. Fujimori, H. Kaneko,

T. Fukuzumi and M. Noguchi, *Agric. Biol. Chem.*, **42**, 327 (1978).
2) C. C. J. Culvenor, *Tetrahedron Lett.*, **1966**, 1091.
3) T. Fujimori, R. Kasuga, H. Kaneko and M. Noguchi, *Agric. Biol. Chem.*, **39**, 913 (1975).
4) K. Mori, *Tetrahedron*, **30**, 1065 (1974).
5) A. J. Aasen, S.-V. Almqvist and C. R. Enzell, *Beitr. Tobakforsch.*, **8**, 366 (1976).
6) I. Wahlberg, K. Karlsson, D. J. Austin, N. Junker, J. Roeraade and C. R. Enzell, *Phytochemistry*, **16**, 1217 (1977).
7) E. Demole and D. Berthet, *Helv. Chim. Acta*, **55**, 1866 (1972).
8) H. H. Strain, *J. Am. Chem. Soc.*, **70**, 1672 (1948).
9) D. E. Loeber, S. W. Russell, T. P. Toube, B. C. L. Weedon and (in part) J. Diment, *J. Chem. Soc. C*, **1971**, 404.