Note

Phytuberol, from Japanese Domestic Tobacco, *Nicotiana tabacum* cv. Suifu*Yoshikazu TAKAGI, Takane FUJIMORI,
Hajime KANEKO and Kunio KATOCentral Research Institute, The Japan Tobacco
Public Corporation, Umegaoka
6-2, Midori-ku, Yokohama 227

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We have described the isolation of several new flavor constituents of tobacco such as 1,4-epoxy-2,10-dihydroxy-7-isopropyl-4-methyl-5*E*-undecene¹⁾ and 3-hydroxy-5,6-epoxy- β -ionol²⁾ from Japanese domestic tobacco, *Nicotiana tabacum* cv. Suifu. We report here the isolation and identification of phytuberol and its derivatives from Suifu tobacco.

A methanol eluate from a silicic acid column chromatography of neutral volatiles of Suifu tobacco was designated as Fraction-11.¹⁾ Rechromatography of Fraction-11 was carried out using ether-methanol mixture with a stepwise increase of methanol concentration and Fraction-11-1 (ether/methanol=95: 5) having good flavor was obtained. Compound (1) was found in Fraction-11-1 and isolated as 2.5 mg of a slight yellow oil by preparative gas liquid chromatography. The mass spectrum showed significant peaks at m/e 252 (M^+ , 22%), 223 (31), 207 (50), 205 (100), 163 (31), 149 (70), 109 (33), 107 (52), 95 (62), 93 (41), 59 (45), 43 (81), 41 (35). By the high resolution MS, the molecular formula of (1) was estimated as $C_{16}H_{26}O_4$ (found: 252.1740, calcd.: 252.1726). In the IR spectrum, bands were showed at 3450 cm^{-1} , 2950, 1622, 1468, 1460, 1379, 1153, 1085, 1040, 913, 862, 813, 800 and 736. The NMR spectrum (100 MHz, $CDCl_3$) showed a $>C-CH_3$ group (δ 1.01, s, 3H), a $-C(CH_3)_2-OH$ group (δ 1.21, s, 6H), a $>C-CH_3$ group (δ 1.56, s, 3H), a $-O-CH_2-C<$ group (δ 3.26, d, 1H, $J=8.8\text{ Hz}$; δ 3.40, d, 1H, $J=8.8\text{ Hz}$; signals of AB system) and a *cis*- $CH=CH-$ group (δ 4.66, d, 1H, $J=2.7\text{ Hz}$; δ 6.43, d, 1H, $J=2.7\text{ Hz}$; irradiation at δ 4.66 simplified the doublet at δ 6.43). These spectroscopic data of (1) were identical with those of phytuberol which have been isolated as a major stress metabolite produced by infected potato tubers.³⁾ Further, we have isolated two compounds, 4.2 mg of (2) and 3.4 mg of (3), from the same fraction (Fraction-11-1). The precise mass determination established the molecular formula of (2)

as $C_{16}H_{26}O_4$ and a fragment of the highest mass of (3) as $C_{16}H_{24}O_3$ ($M^+ - 32$). NMR signals of (2) and (3) had a strong resemblance to those of phytuberol (1) except the disappearance of olefinic signals and the newly occurring 3H singlet at δ 3.36 in (2) and δ 3.42 in (3) which showed the existence of a methoxyl group. These data indicated that they were 2-methoxydihydrophytuberol and their coupling system between 2H at C-3 and H at C-2 implied that (2) was 2 α - and (3) was 2 β -methoxydihydrophytuberol¹⁾ (see Table I).

TABLE I.

	(1)	(2)	(3)
NMR (δ^{CDCl_3} TMS)	1.01, s, 3H 1.21, s, 6H 1.56, s, 3H 3.26, d, 1H ($J=8.8$) 3.40, d, 1H ($J=8.8$) 4.66, d, 1H ($J=2.7$) 6.43, d, 1H ($J=2.7$)	1.03, s, 3H 1.22, s, 6H 1.59, s, 3H 3.40, d, 1H ($J=8.8$) 3.52, d, 1H ($J=8.8$) 1.98, dd, 1H ($J=2.5, 14.0$) 2.34, dd, 1H ($J=5.5, 14.0$) 4.94, dd, 1H ($J=2.5, 5.5$) 3.36, s, 3H	1.08, s, 3H 1.22, s, 6H 1.52, s, 3H 3.51, d, 1H ($J=8.8$) 4.01, d, 1H ($J=8.8$) 2.22, d, 2H ($J=3.5$) 4.94, t, 1H ($J=3.5$)
IR (cm^{-1})	3450, 2950, 1622, 1468, 1460, 1379, 1153, 1085, 1040, 913, 862, 813, 800, 736	3400, 2950, 1660, 1593, 1452, 1384, 1365, 1312, 1284, 1247, 1188, 1123, 1051, 1037, 999, 932, 900	3410, 2965, 1718, 1676, 1637, 1453, 1384, 1330, 1252, 1200, 1168, 1061, 1020, 971, 914, 821, 726
MS (m/e)	205 (100), 43 (81), 149 (70), 107 (52), 207 (50), 93 (41), 41 (35), 109 (33), 163 (31), 223 (31), 252 (M^+ , 22)	101 (100), 166 (64), 43 (42), 208 (37), 109 (35), 121 (35), 193 (33), 175 (31), 151 (31), 284 (M^+ , 1)	43 (100), 125 (64), 107 (28), 81 (27), 101 (26), 109 (23), 41 (22), 55 (17), 208 (16), 252 ($M^+ - 32$, 0.5)

* Japanese Domestic Tobacco Flavor. Part III.

When Fraction-11-1 was rechromatographed on silicic acid using ether-methanol mixture, it was observed by gas liquid chromatography that (2) and (3) increased, but little phytuberol could be detected in the eluates. From this fact they would arise from the silicic acid catalysed addition of methanol across the 2,3-double bond of (1) because 2 α - and 2 β -ethoxydihydrophytuberin have been obtained from a silicic acid catalyzed addition of ethanol.³⁾ Hence (2) and (3) seems to have the strong possibility of artifacts of (1) during the process of isolation and purification. Optical rotation of (1) could not be measured owing to the small amount of sample. This is the first report of the natural existence of phytuberol structure in *N. tabacum*.

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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) Y. Takagi, T. Fujimori, H. Kaneko, T. Fukuzumi and M. Noguchi, *Agric. Biol. Chem.*, **42**, 1785 (1978).
 - 3) D. T. Coxon, K. R. Price, B. Howard and R. F. Curtis, *J. Chem. Soc. Perkin I.*, 53 (1977).
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