Tetrahedron Letters,Vol.26,No.45,pp 5551-5554,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

THREE FUNGITOXIC CYCLOPENTANOID SESQUITERPENES FROM STROMATA OF EPICHLOE TYPHINA

Teruhiko Yoshihara*, Satoshi Togiya, Hiroyuki Koshino, Sadao Sakamura*
Department of Agricultural Chemistry, Faculty of Agriculture,
Hokkaido University, Sapporo 060, Japan
Tadayuki Shimanuki
National Grassland Research Institute, Nishinasuno 329-27, Japan
Tohru Sato, Akitoshi Tajimi
Hokkaido National Agricultural Experiment Station, Sapporo, Japan

Summary: New fungitoxic sesquiterpenes, chokol A (1), B (2), and C (3), have been isolated from stromata of Epichloe typhina. Their structures and the relative configuration of 3 have been also elucidated from spectral data.

A phytopathogenic fungus, Epichloe typhina, invades timothy plant (Phleum pratense) and produces a peculiar shape of the stroma at the stalk like a cattail which has been named choke. It is generally accepted that infection of the plants by the pathogen causes not only formation of the choke but systemic effect to the plants through the endohyphae. The timothy infected by this fungus has been found to be resistant against another pathogen, Cladosporium phlei.[1] Our interest has been directed to isolate the biologically active compounds, especially fungitoxins, from chokes with the intention of revealing the chemical interaction between fungus and plant. This communication deals with the isolation and identification of three new fungitoxins named chokol A (1), B (2), and C (3).

n-Hexane soluble fraction from 70% ethanol extract of chokes (2.5 Kg) collected from the infected plants was chromatographed on silica gel and Sephadex LH-20 columns. TLC bioautography[2] with Cladosporium herbarum was employed to monitor the activity.

Chokol A (1), approx. 20 mg, showed $[\alpha]_D^{22}$ -26.6° (c 1.0, EtOH) and a molecular formula $C_{12}H_{22}O_2$ from the high resolution MS m/z 198 (198.1628, Calcd. 198.1620). The IR spectrum exhibited the presence of hydroxyl (3350, 1150, 1050 cm^{-1}) and terminal methylene groups (3050, 1700~1780, 1640, 880 cm^{-1}). The ^{1}H-NMR spectrum showed the presence of two methyls

(δ 0.87, d, J=6.8 Hz and 1.28, s), which was confirmed by ^{13}C-NMR spectrum (δ 10.7 and 26.5 ppm). And other ten carbons were grouped into four classes; five methylenes (δ 28.8, 30.2, 31.1, 39.9, 62.7 ppm), two methines (δ 47.6, 51.7 ppm), one quarternary (δ 80.3 ppm) and a pair of double bond (δ 108.2 and 151.3 ppm).

Treatment of chokol A (1) with acetic anhydride-pyridine gave the corresponding monacetate (4), FI-MS M^+240, in the ^{1}H-NMR spectrum of which the singnal at 3.67 (t) shifted downfield (δ 4.08). Since compound 4 has still the absorption due to a hydroxyl group (3450, 1030 cm^{-1}) and shows a dehydration peak, m/z 222 (M^+-H_2O), the remaining one oxygen atom must be assigned to a tertiary hydroxyl group. Detailed ^{1}H-NMR study of 1 with spin decoupling experiments led to three fragments I~III as shown in Fig. 1, in which the spectral characteristics for structure determination are summarized. Thus a planar structure of chokol A was elucidated to be 4-(3-hydroxy-2,3-dimethylcyclopentyl)-pent-4-ene-1-ol (1).

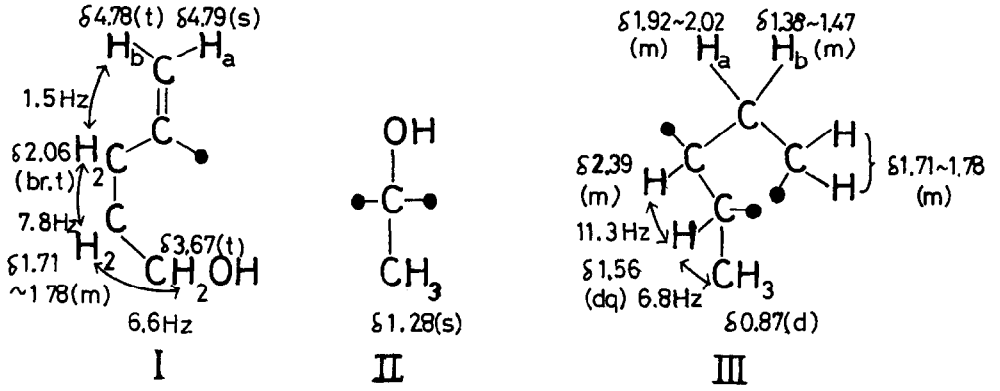

Fig. 1

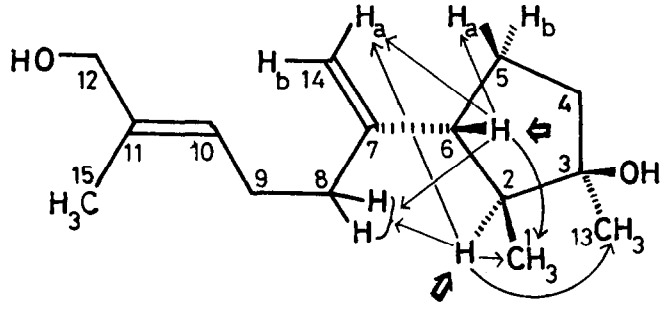

Fig.2

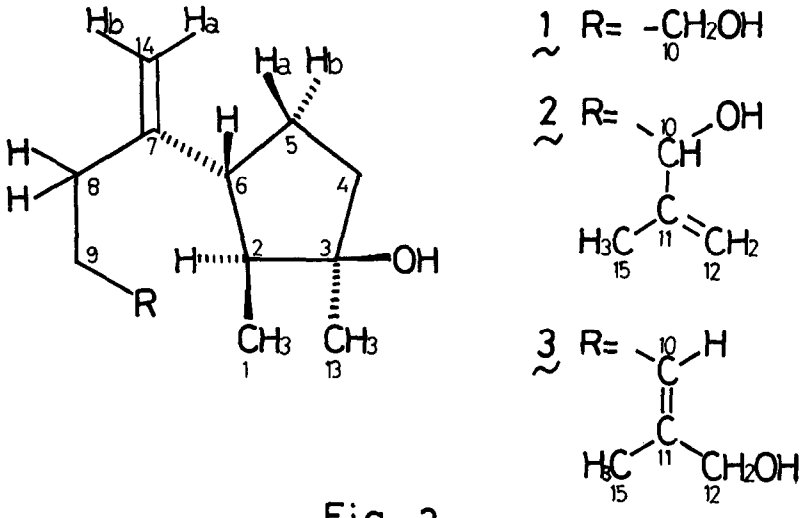

Fig. 3

Chokol B (2), approx. 20 mg, was obtained as sirup of molecular formula $C_{15}H_{26}O_2$ (M^+238). It showed ^{1}H-NMR spectral features[3] similar to those of 1 but typical signals due to isopropenyl [δ 1.74(-CH_3, s), 4.85 and 4.95 ($>C=C\langle^{H}_{H}$)] and methine [4.09(t)] were detectable instead of hydroxymethyl [3.67(t)]. Acetylation with acetic anhydride-pyridine afforded the monoacetate (5) which still contained a free OH (3400 cm^{-1}). The signal of a methine proton shifted downfield (δ 5.1~5.3). To account for these data the structure of chokol B was assigned to 6-(3-hydroxy-2,3-dimethylcyclopentyl)-2-methylhept-1,6-diene-3-ol (2).

Chokol C (3), approx. 14 mg, showed mp 51.0~53.0°C, $[\alpha]_D^{22}$-32.0°(c 0.25, EtOH) and a molecular formula of $C_{15}H_{26}O_2$, i.e. isomeric with 2. In the ^{1}H-NMR spectrum[4] it contained a vinylic proton (δ 5.43 ppm, tq) and a hydroxymethyl (δ 4.00) but lacked the resonances associated with vinylic protons of an isopropenyl and a methine. Based on the above spectral properties the structure of chokol C was shown as 6-(3-hydroxy-2,3-dimethylcyclopentyl)-2-methylhept-2,6-diene-1-ol (3). The double bond was assigned to the E geometry since the ^{13}C resonance[4] of the primary alcohol appeared at 68.8 ppm (E 68.1,[5] 68.8,[6] Z 60.5[5] ppm).

The relative stereochemistry of 3 was clarified by nuclear Overhauser effect(NOE) difference spectroscopy (Fig. 2). Irradiation of 2-H increased the intensity of the signals due to 1-H, 13-H, 14-Ha and 8-H. Also, irradiation of 6-H increased the intensity of the signals due to 1-H, 5-Ha, 14-Ha and 8-H. These observations surely support the relative configuration of chokol C (3) as depicted in Fig. 3.

To our knowledge there have been reports on cyclopentanoid sesquiterpenes with the same configuration as that of 3;[6,8] cyclonerodiol from Trichothesium sp.[7] and Gibberella fujikuroi[9,10] and cyclonerotriol from Fusarium culmorum.[6] The biosynthesis of chokols is assumed to proceed via an intermediate of nerolidyl pyrophosphate in similar pathway to that of cyclonerodiol.[11] Further the elimination of C_3 unit may occur in case of chokol A (1).

Acknowledgments. The authors are indebted to Professor S. Takao of Hokkaido University for the gift of strains of C. herbarum and Associate Professor S. Tahara of the same university for his helpful suggestion.

REFERENCES AND FOOTNOTES

1) T. Shimanuki and T. Sato, Abstracts of Papers, Annual Meeting of The Phytopathological Society of Japan, Tsukuba, April 1984, p 224.
2) A. L. Homans and A. Fuchs, J. Chromatog., 51, 325(1970).
3) Spectral data for 2: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 0.87 (3H, d, J=6.3Hz), 1.28 (3H, s), 1.38~1.48 (1H, m), 1.56 (1H, ddq, J=1.9, 6.8, 11.2Hz), 1.63~1.78 (4H, m), 1.74 (3H, s), 1.92~2.02 (2H, m), 2.09 (1H, dddd, J=1.0, 5.9, 6.4, 9.3Hz), 2.39 (1H, ddddd, 1.0, 2.0, 8.8, 9.3, 11.2Hz), 4.09 (1H, dd, J=5.9, 6.8Hz), 4.78 (1H, d, J=1.0Hz), 4.79 (1H, d, J=1,0Hz), 4.85 (1H, br. s), 4.95 (1H, br.s); ^{13}C-NMR (50.2 MHz, $CDCl_3$), 10.7, 17.6, 26.6, 28.8, 30.0, 33.5, 40.0, 47.7, 51.9, 75.8, 80.3, 108.2, 110.0, 147.6, 151.5.
4) Spectral data for 3: ^{1}H-NMR (500 MHz, $CDCl_3$) 0.87 (3H, d, J=7.0Hz), 1.28 (3H, s), 1.37~1.47 (1H, m), 1.55 (1H, dq, J=7.0, 11.9Hz), 1.68 (3H, s), 1.76 (2H, dd, J=7.6, 8.2Hz), 1.93~1.99 (1H, m), 2.05 (2H, dt, J=3.7, 7.8Hz), 2.20 (2H, dt, J=7.0, 7.3Hz), 2.39 (1H, ddd, J=9.2, 9.2, 11.3Hz), 4.00 (2H, s), 4.77 (1H, d, J=1.5Hz), 4.79 (1H, s), 5.43 (1H, dt, J=1.2, 7.0Hz); ^{13}C-NMR (50.2 MHz, $CDCl_3$), 10.7 (q), 13.8 (q), 26.4 (t), 26.6 (q), 28.7 (t), 33.6 (t), 40.0 (t), 47,6 (d), 51.8 (d), 68.8 (t), 80.3 (s), 108.2 (t), 125.9 (d), 134,9 (s), 151.4 (s).
5) J. B. Stothers, Carbon-13 NMR Spectroscopy, Academic Press, New York, 1972, p 188.
6) J. R. Hanson, P. B. Hitchcock, and R. Nyfeler, J. Chem. Soc., 1975, 1586.
7) S. Nozoe, M. Goi, and N. Morisaki, Tetrahedron Lett., 1970, 1293.
8) D. E. Cane and R. Lyengar, Tetrahedron Lett., 1977, 3511.
9) B. E. Cross, R. E. Markwell, and J. C. Stewart, Tetrahedron 27, 1663(1971).
10) D. W. Pitel, G. P. Arsenault, and L. C. Vining, J. Antibiot., 24, 483(1971).
11) D. E. Cane and M. S. Shiao, J. Am. Chem. Soc., 100, 3203(1978).

(Received in Japan 3 September 1985)