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Odorous Metabolites of Actinomyces Biwako-C and -D Strain isolated from the Bottom Deposits of Lake Biwa. Identification of Geosmin, 2-Methylisoborneol, and Furfural

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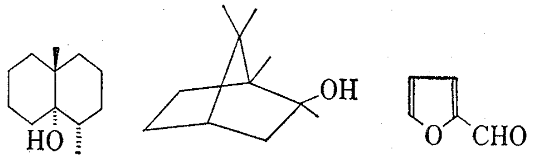
In the summer of 1969, an earthy-musty odor and taste occurred in water supplies of Kyoto City, and since then this unpleasant odor and taste appeared repeatedly every year. In order to clarify the origin of this odor and taste, we have commenced the study on odorous metabolites produced by actinomycetes and algae grown in the Southern Basin of Lake Biwa, which is the source of public water supplies of Kyoto, Osaka, Kobe, and surrounding cities.

In previous papers,[2)] we reported the isolation and identification of geosmin (I)[3)] and 2-methylisoborneol (II)[4)] from an actinomyces Biwako-B strain (a variant of *Streptomyces resistomycificus*) which was obtained from the bottom deposits of Lake Biwa. In 1971, we isolated two other strains of actinomyces, Biwako-C and Biwako-D strain, which have been identified as a variant of *Streptomyces albosporeus* and a variant of *Streptomyces filipinensis*, respectively. Present paper deals with the odorous metabolites produced by them.



I II III

Chart 1

Experimental

Separation of Odorous Metabolites of Actinomyces Biwako-C Strain——Biwako-C strain was isolated from the bottom deposits collected in the offing about 100 m apart from the mouth of Ogoto River.

Biwako-C strain was streaked on Krainsky agar medium and the culture plates (90 Petri dishes (10 cm *i.d.*)) were incubated at 28° for 12 days. Sterilized distilled water (10 liters) was added to the combined entire culture medium and the whole material was slowly distilled until about 20% of its volume was collected as a distillate. The distillate was further concentrated by additional two distillations and the final distillate (100 ml) was saturated with sodium chloride, extracted three times with methylene chloride. After drying over anhydrous magnesium sulfate, methylene chloride was carefully evaporated to give a minute amount of earthy-smelling oil.

Separation of Odorous Metabolites of Actinomyces Biwako-D Strain——Biwako-D strain was isolated from the bottom deposits collected in the offing about 50 m apart from Sakamoto Harbor. This actinomyces was cultured and its volatile metabolites were collected in the same manner as given for Biwako-C strain, whereupon was obtained a small amount of oily substance with an earthy-putrid odor.

Gas Chromatography (GC)——GC was carried out on a 10% PEG 20 M column (stainless steel 2 m × 3 mm *i.d.*, on 60—80 mesh chromosorb WNAW) at 150°, a 10% SE-30 column (stainless steel 1 m × 3 mm

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2) T. Kikuchi, T. Mimura, Y. Moriwaki, K. Negoro, S. Nakazawa, and H. Ono, *Yakugaku Zasshi*, **91**, 1255 (1971); *idem*, *ibid.*, **92**, 652 (1972); *idem*, *ibid.*, **93**, 658 (1973).

3) N.N. Gerber, *Tetrahedron Letters*, **1968**, 2971.

4) L.L. Medsker, D. Jenkins, and J.F. Thomas, *Environ. Sci. Technol.*, **3**, 476 (1969); N.N. Gerber, *J. Antibiotics* (Tokyo), **22**, 508 (1969).

i.d., on 60—80 mesh chromosorb WNAW) at 120°, and a 15% Reoplex 400 column (stainless steel 2 m × 3 mm *i.d.*, on 60—80 mesh chromosorb WNAW) at 150° with a Hitachi Gas Chromatograph Model 063 equipped with a hydrogen flame ionization detector. The carrier gas was nitrogen or helium (30 ml/min).

Mass Spectrometry combined with Gas Chromatography (GC-MS)——A Hitachi Mass Spectrometer Model RMU-6E was combined with a Hitachi Gas Chromatograph Model K-53, and a Watson-Biemann type molecular separator was used as the carrier gas separator. GC was performed on a 25% PEG 20M column (glass tube 1.5 m × 2 mm *i.d.*, on 60—80 mesh chromosorb W) at 115°, using helium (2 kg/cm^2) as the carrier gas. Mass spectra were measured under the following condition; ionization voltage: 70 eV, accelerator voltage: 1800 V; ion source temp.: 220°.

Result and Discussion

Biwako-C strain produced a strong earthy odor upon pure culture with Krainsky agar medium. As described in experimental part, steam distillation of the whole culture and extraction of the distillate with methylene chloride gave a small amount of oily substance having the earthy odor.

Gas chromatographic examination of this substance using three kinds of columns (10% PEG 20M, 10% SE-30, and 15% Reoplex 400) indicated the presence of a compound which has the same retention time as *dl*-geosmin (I). Among the gas chromatograms obtained, one example on a 15% Reoplex 400 column is reproduced in Fig. 1a, in which the peak A corresponds to geosmin (I). Furthermore, GC–MS of this oily substance confirmed the identity of peak A with geosmin (I).

On the other hand, pure culture of Biwako-D strain and extraction of the entire culture gave a small amount of oily substance with an earthy-putrid odor.

GC of this oil under three conditions as given above revealed the peaks corresponding to geosmin (I) and 2-methylisoborneol (II), the latter being predominant. Fig. 1b shows the gas chromatogram taken on a 15% Reoplex 400 column, where the peaks A and B correspond to geosmin (I) and 2-methylisoborneol (II), respectively. In addition, an intense peak (peak C) is observed at the retention time 2.2 min.

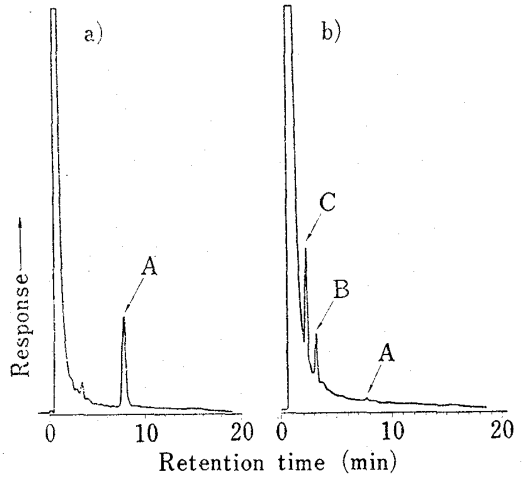

Fig. 1. a) Gas Chromatogram of the Odorous Metabolites of Biwako-C Strain from Lake Biwa
b) Gas Chromatogram of the Odorous Metabolites of Biwako-D Strain from Lake Biwa

condition: column, 15% Reoplex 400 on chromosorb WNAW; column temp., 150°; carrier gas, N_2 or He (30 ml/min)

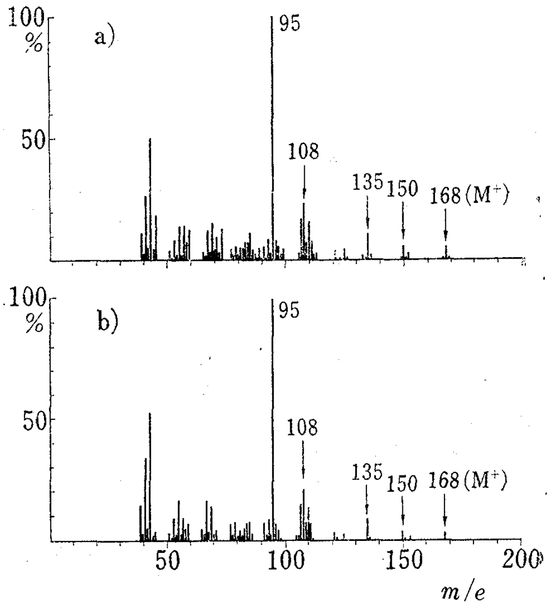

Fig. 2. a) Mass Spectrum of an Odorous Metabolite (Peak B) of Biwako-D Strain
b) Mass Spectrum of Authentic 2-Methylisoborneol

Then, we examined the GC–MS of this substance.[5] As shown in Fig. 2, the mass spectrum arising from peak B is identical with that of authentic 2-methylisoborneol (II) measured under the same condition.

The mass spectrum arising from peak C is characterized by the abundant molecular ion peak at *m/e* 96 and the abundant M-1 ion peak at *m/e* 95. This suggests that the compound might be an aldehyde,[6] having a molecular formula $C_5H_4O_2$ or C_6H_8O. Other significant fragment peaks appear at *m/e* 67, 39, and 29. This spectral pattern is in good agreement with that of furfural (III) reported already.[7] Eventually, identity of the peak C with furfural was fully proved by the direct comparison with the mass spectrum of authentic furfural (III) taken under the same condition, as illustrated in Fig. 3.

Furfural (III) has been reported as an odorous metabolite of an alga, *Synura petersenii*,[8] but our present result is the first example of identification of furfural (III) from the actinomyces.

From the above observations, it appears that the aquatic actinomycetes produce several kinds of odorous metabolites and these compounds contribute jointly to the objectionable odor and taste in public water supplies.

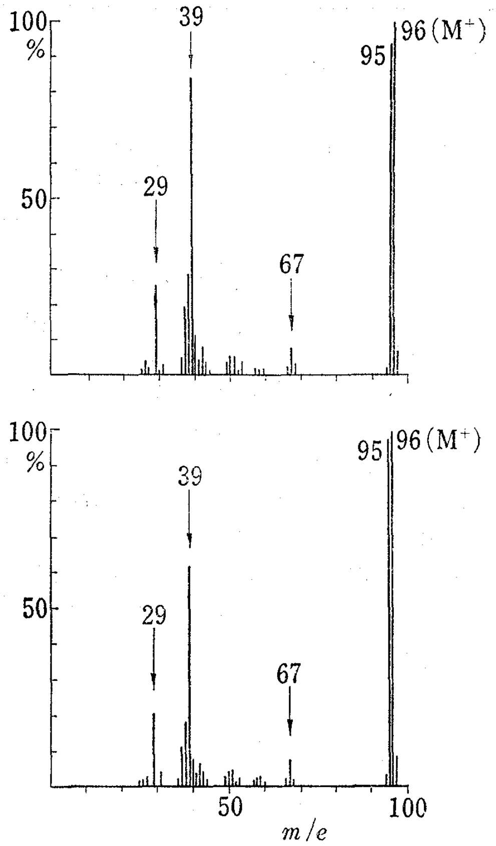

Fig. 3. a) Mass Spectrum of the Other Metabolite (Peak C) of Biwako-D Strain
b) Mass Spectrum of Authentic Furfural

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5) The peak A in this case was too weak to give a significant mass spectrum.

6) R.M. Silverstein and G.C. Bassler, "Spectrometric Identification of Organic Compounds," 2nd ed., John Wiley and Sons, Inc., New York, 1967, p. 21.

7) Q.N. Porter and J. Baldas, "Mass Spectrometry of Heterocyclic Compounds," John Wiley and Sons, Inc., New York, 1971, p. 114.

8) R.P. Collins and K. Kalnins, *Lloydia*, 28, 48 (1965).