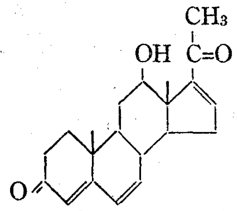
by the double-doublet lines at 3.71 ppm with coupling constants of 5 and 10 Hz, due to a carbinol methine proton.

When NA was subjected to catalytic hydrogenation over Pd/charcoal, two hexahydro derivatives, NA-I (mp 173—175°, Calcd. for $C_{21}H_{32}O_3$ 332.2351; Found 332.2334) and NA-II (mp 157—160°, Found 332.2324) were obtained. NA-II, on oxidation with CrO_3, gave a triketone compound (mp 208—212°, $[\alpha]_D+$ 174°, Calcd. for $C_{21}H_{30}O_3$ 330.2195; Found 330.2161), which was identified as 5α-pregnane-3,12,20-trione on direct comparison with the authentic sample synthesized from hecogenin.[3] The structure of NA, therefore, was elucidated as 12β-hydroxypregna-4,6,16-triene-3,20-dione.


neridienone A

Recently, 12β-hydroxypregna-4,6-diene-3,20-dione,[4] the dihydro-derivative of NA, and the several C_{21}-steroids[5] have been known to possess the peculiar effect against fish as the defence substance of the water beetles. In our experiments on NA, goldfishes were observed to go down to the bottom of the vessel and keep their bodies static within 10 min at the concentration of *ca.* 10 mg/liter.

In the early studies on the cardiac glycosides of *Nerium* by Reichstein and his associates, the presence of the substances having the absorptions at *ca.* 280 and 240 nm was noted as "Substance 1" in the bark of *N. odorum*[6] and "Substance A" in the seeds of *N. oleander*,[7] respectively, although no further investigations have been provided. NA is most likely the same or the closely related substance as those obtained by them.

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[3] R.B. Wagner, J.A. Moore and R.F. Forker, *J. Am. Chem. Soc.*, **72**, 1856 (1950).
[4] M.C. Chadha, N.K. Joshi, V.R. Mamdapur and A.T. Sipahimalani, *Tetrahedron*, **26**, 2061 (1970).
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[7] H. Jäger, O. Schindler and T. Reichstein, *Helv. Chim. Act.*, **42**, 976 (1959).

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UDC 547.572.02 : 547.568.1.02 : 615.36.011.5

Odorous Metabolites of Aquatic Actinomycetes. Identification of 1-Phenyl-2-propanone and 2-Phenylethanol[1]

Investigations on odorous metabolites produced by actinomycetes have been greatly advanced in the last decade by the use of gas chromatography (GC) and mass spectrometry combined with GC (GC–MS). In 1965, Gerber, *et al.*[2] isolated a strongly earthy-smelling substance, geosmin, from seventeen strains of *Streptomyces*, *Nocardia*, and *Microbispora*

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[1] This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, which is gratefully acknowledged.
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species. In 1969, Medsker, *et al.*[3] and Gerber[4] reported the isolation and characterization of 2-methylisoborneol with a camphor-menthol-like odor from several actinomycetes. These two compounds may be responsible for the unpleasant earthy-musty odor and taste occurred in public water supplies.[5] Many other volatile metabolites of actinomycetes were reported.[6]

Recently we isolated two smelling actinomycetes, tentatively named Kashiwai-D strain and Nunobiki-11 strain, from the bottom deposits of the grit chamber of the Inbanuma Water Intake Plant, Chiba Prefectural Water Works Bureau and the bottom deposits of Nunobiki Reservoir, Kobe City Water Supply Bureau, respectively, and their odorous metabolites were examined.

Kashiwai-D strain, which closely resembles *Streptomyces platensis*,[7] was cultured on Krainsky agar medium (79 Petri dishes (10 cm i.d.)) at 28° for 10 days and its volatile metabolites were collected in the same manner as described in a previous paper,[8] whereupon was

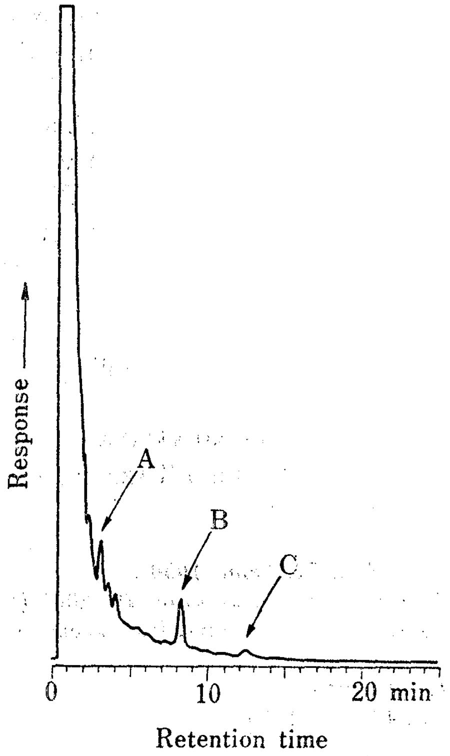

Fig. 1. Gas Chromatogram of the Odorous Metabolites of Kashiwai-D Strain

condition: Hitachi Gas Chromatograph Model 063; column, 10% PEG 20M on Chromosorb WNAW (2m × 3mm i.d. stainless steel); detector, flame ionization detector; column temp., 150°; carrier gas, N_2(30 ml/min)

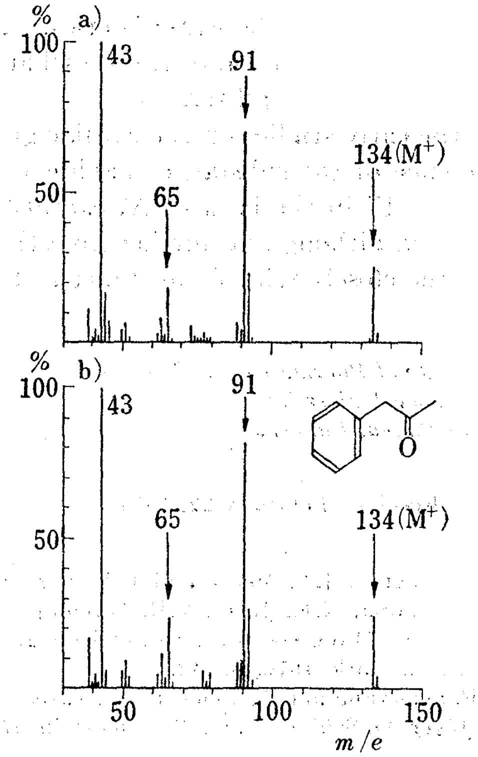

Fig. 2. a) Mass Spectrum of the Odorous Metabolite (Peak B) of Kashiwai-D Strain
b) Mass Spectrum of Authentic 1-Phenyl-2-propanone

condition: GC column, 25% PEG 20M on Chromosorb W (1.5m × 2mm i.d. glass tube); column temp., 115°; carrier gas, He (2.2kg/cm^2); MS ionization voltage, 70 eV; accelerator voltage, 1800 V; carrier gas separator, Watson–Biemann type molecular separator (Hitachi Mass Spectrometer RMU-6E)

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3) L.L. Medsker, D. Jenkins, and J.F. Thomas, *Environ. Sci. Technol.*, **3**, 476 (1969).
4) N.N. Gerber, *J. Antibiotics* (Tokyo), **22**, 508 (1969).
5) A.A. Rosen, C.I. Mashni, and R.S. Safferman, *Water Treatment and Examination*, **19**, 106 (1970); T. Kikuchi, T. Mimura, and Y. Moriwaki, *Yakugaku Zasshi*, **92**, 1441 (1972); T. Kikuchi, T. Mimura, Y. Masada, and T. Inoue, *Chem. Pharm. Bull.* (Tokyo), **21**, 1847 (1973).
6) T. Kikuchi, *Japan Analyst* (*Bunseki Kagaku*), **22**, 1530 (1973) and references cited therein.
7) Identification of this actinomycete was carried out by Dr. M. Hamada of Institute of Microbial Chemistry, which is gratefully acknowledged.
8) T. Kikuchi, T. Mimura, Y. Itoh, Y. Moriwaki, K. Negoro, Y. Masada, and T. Inoue, *Chem. Pharm. Bull.* (Tokyo), **21**, 2339 (1973).

obtained a small amount of oily substance with an earthy-putrid odor. Gas chromatographic examination of this oil using a 10% Carbowax (PEG) 20M column indicated the presence of furfural (peak A)[8] and geosmin (peak C) as illustrated in Fig. 1. The above oily substance was then submitted to silica gel column chromatography (0.8×15 cm) and elution with ether–pentane (1: 99) gave an oil[9] having a strong earthy odor. Subsequent elution with methylene chloride afforded a small amount of oily substance having an aromatic odor, which showed the peaks A and B in GC.

The mass spectrum arising from the peak B in GC–MS showed the molecular ion peak at *m/e* 134 ($C_9H_{10}O$)[10] and the base peak at *m/e* 43 as shown in Fig. 2a. The peaks at *m/e* 91 and 65 suggested the presence of a benzyl group.[11] Therefore, the compound corresponding to the peak B was supposed to be 1-phenyl-2-propanone and this was proved by GC–MS comparison with an authentic sample as shown in Fig. 2.

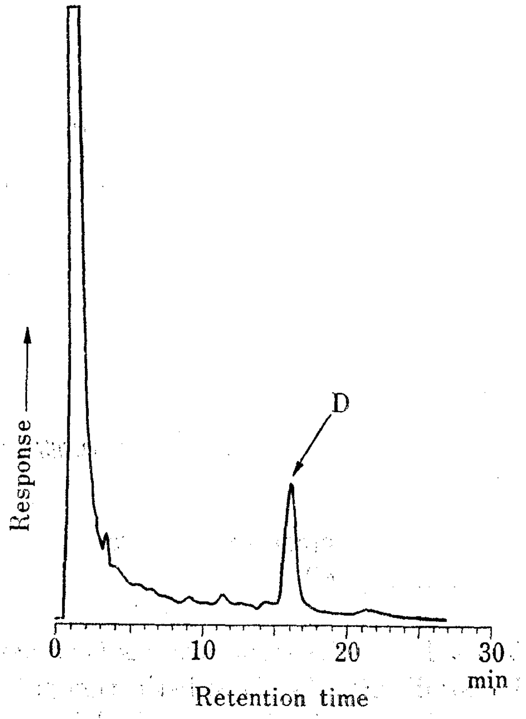

Fig. 3. Gas Chromatogram of the Odorous Metabolites of Nunobiki-11 Strain

condition: the same as described in the legend to Fig. 1

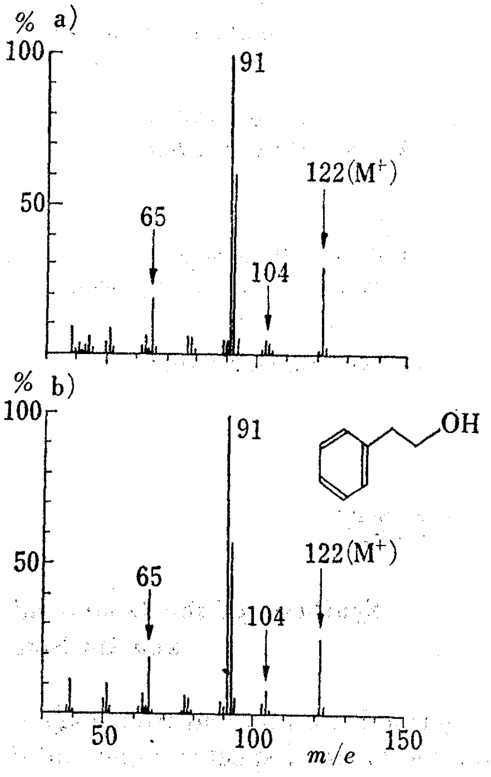

Fig. 4. a) Mass Spectrum of the Odorous Metabolite (Peak D) of Nunobiki-11 Strain
b) Mass Spectrum of Authentic 2-Phenylethanol

condition: the same as described in the legend to Fig. 2

Nunobiki-11 strain was cultured with soy bean meal medium[12] (12 liters) or Krainsky agar medium (200 Petri dishes (10 cm i.d.)) at 27° for 7 days. The whole culture was then treated in the same manner as above to give a small amount of oily substance having an aroma-

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[9] This oily substance showed the peak C corresponding to geosmin in GC.

[10] Alternative possibility of $C_{10}H_{14}$ could be excluded in consideration of the retention time in GC.

[11] H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Mass Spectrometry of Organic Compounds," Holden-Day, Inc., San Francisco, 1967, p. 72.

[12] Soy bean meal medium consisted of 10 g of soy bean meal, 10 g of peptone, 20 g of starch, and 5 g of sodium chloride in 1 liter of distilled water.

tic odor, which revealed practically single peak (peak D) in GC (Fig. 3).

The mass spectrum (Fig. 4a) of the peak D obtained by GC–MS method showed the molecular ion peak at *m/e* 122 ($C_8H_{10}O$) and the base peak at *m/e* 91. Other significant peaks appeared at *m/e* 65 and at *m/e* 104 (M−18). From this result, the peak D was considered to correspond to 2-phenylethanol. This was confirmed by the comparison with the mass spectrum of authentic 2-phenylethanol obtained under the same condition (Fig. 4).

Neither 1-phenyl-2-propanone nor 2-phenylethanol is badly smelling, but these compounds may be partly responsible for the unpleasant odor and taste in public water supplies in combination with geosmin and 2-methylisoborneol.

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Synthesis of the Compound proposed as the Structure of Lagerine and Its Non-identity with Lagerine[1]

Lagerine is an alkaloid from *Lagerstroemia indica* L. and its structure has been assigned as I on the basis of spectroscopic and degradative studies.[2] As the substitution pattern of the ring C in the structure (I) differs from those of all other Lythraceae alkaloids hitherto isolated,[3] the synthesis of I was performed to corroborate the structure of lagerine.

Condensation of *o*-vanillin (II) with isopelletierine (III)[4] in aqueous sodium hydroxide afforded the quinolizidin-2-one (IV) [*m/e*: 275 (M^+), $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 1721 (C=O)] and the half-acetal (V) [*m/e*: 275 (M^+), $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 3570 (OH)] in the ratio of 1: 3. On treatment with aqueous sodium hydroxide, the both compounds were interconverted to each other. Acetylation of IV and V with acetic anhydride in pyridine gave the *trans*-quinolizidin-2-one (VI) [*m/e*: 317 (M^+), $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 2790, 2760 (Bohlmann bands), 1762 (OAc), 1720 (C=O), δ: 3.45 (1H, d-d, $J=12$; 3.5 Hz, C$\underline{H}$Ar)] and the *cis*-quinolizidin-2-one (VII) [*m/e*: 317 (M^+), $\nu_{\max}^{\mathrm{CHCl_3}}$ cm^{-1}: 1765 (OAc), 1710 (C=O), δ: 4.53 (1H, d-d, $J=6.5$; 4 Hz, C$\underline{H}$Ar)], respectively.

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[1] Presented at the 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April, 1974.

[2] J.P. Ferris, R.C. Briner and C.B. Boyce, *J. Am. Chem. Soc.*, **93**, 2958 (1971).

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