UDC 581.13:582.288

Shinsaku Natori[*1] and Hidejiro Nishikawa[*2]: Structure of Compound G, a Metabolite of *Oospora sulphurea-ochracea* v. Beyma.[*3]

(*Faculty of Pharmaceutical Sciences, University of Tokyo,*[*1] *and Department of Agriculture and Veterinary Medicine, Nihon University*[*2])

In the previous paper,[1] we reported the structures of compounds A, C, D, E[*4] and F isolated as the metabolites of *Oospora sulphurea-ochracea.*[2~7] Prior to this, the structure of sulochrin (compound B), the major metabolite of the mold, had been established as a benzophenone derivative (I, R^1:R^3:CH_3, R^2:R^4:H).[4,5,7]

In the present paper, compound G, the seventh metabolite[3] of *Oospora sulphurea-ochracea*, whose structure has been remained unsettled, is now proved to be desmethylsulochrin (I, R^1:R^2:R^4:H, R^3:CH_3).

Compound G is pale yellow needles of m.p. 205~207° (with effervescence), whose analytical figures reveal the molecular formula, $C_{16}H_{14}O_7$, and the presence of one each of carboxyl and methoxyl group. Its ultraviolet absorption (Fig. 1) is superimposable with that of sulochrin. Treatment of compound G with diazomethane in acetone gave the methyl ester methyl ether, which was identical with sulochrin dimethyl ether (I, R^1:R^2:R^3:R^4:CH_3).[4] Hydrolysis of compound G with sulfuric acid afforded α-resorcillic acid monomethyl ether[4] and *p*-orsellinic acid.[4] When compound G was treated with boiling methanolic potassium hydroxide a hydroxyxanthonecarboxylic acid was formed, which afforded, on sublimation, 3-methyl-1,6-dihydroxyxanthone.[7] These facts indicated that methyl ester of compound G must be identical with sulochrin, and it was actually proved by the methylation of compound G with diazomethane in ether; thus the structure of compound G being represented by (I, R^1:R^2:R^4:H, R^3:CH_3).

Structures of all the seven metabolite of *Oospora sulphurea-ochracea* has now been established; compounds A, C, D, and F (osoic acids) are diphenyl ethers (III, R^1:CH_3, R^2:R^3:H, R^1CH_3,R^2:H, R^3:CH_3CO, R^1:R^2:CH_3, R^3:H; and R^1:R^2:R^3:H), compound E is a

*1 Present Address: National Institute of Hygienic Sciences, Tamagawa-Yoga, Setagaya, Tokyo (名取信策).

*2 Shimouma-3-chome, Setagaya-ku, Tokyo (西川英次郎).

*3 This paper is a supplementary report of the paper entitled "Structures of Osoic Acids and Related Compounds, Metabolites of *Oospora sulphurea-ochracea* v. Beyma." [1]

*4 Added in Proof (July, 20, 1962): Quite recently C. E. Stickings and A. Mahmoodian (private communication) isolated (+)-dechlorogeodin, m.p. 162~166° after careful drying, $[\alpha]_D$ +186° (EtOH), from *Penicillium frequentans* Westling along with sulochrin (compound B), asterric acid (compound A), and related compouds. Since compound E, hydrate, m.p. 147~148°, $[\alpha]_D$ −66°, from *Oospora sulphurea-ochracea*, was proved to be (−)-dechlorogeodin,[1] they must be optical antipodes, which being proved by the identity of infrared spectra. The difference of the size of the optical rotation would be due to partial decomposition or racemization of compound E before the measurement. Reexamination of $[\alpha]$ of compound E by the use of a Rudolf polarimeter showed that compound E is actually levorotatory and the size is greater than originally reported, though an accurate determination with a pure sample was impossible due to the scarcity of the specimen and the mutation of the mold.

1) S. Natori, H. Nishikawa: This Bulletin, **10**, 117 (1962).
2) H. Nishikawa: Bull. Agric. Chem. Soc. Japan, **12**, 47 (1936).
3) *Idem*: *Ibid.*, **13**, 1 (1937).
4) *Idem*: Acta Phytochim., **11**, 167 (1939).
5) *Idem*: Bull. Agric. Chem. Soc. Japan, **16**, 97 (1940).
6) *Idem*: *Ibid.*, **18**, 13 (1942).
7) M. Itahashi, H. Nishikawa, S. Sagisaka: Tohoku J. Agric. Res., **4**, 277 (1955).
8) R. F. Curtis, C. H. Hassall, S. Natori, H. Nishikawa: Chemistry & Industry(London), **1961**, 1360; C. H. Hassall, A. I. Scott: "Recent Develop. ments in the Chemistry of Natural Phenolic Compounds", p. 119(1961).

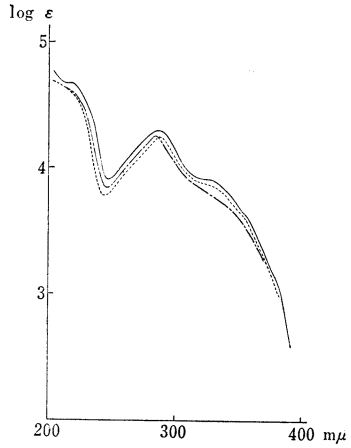
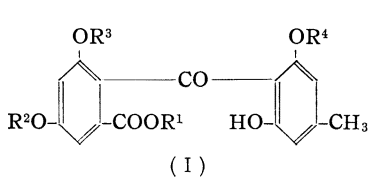


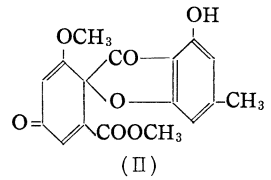
Fig. 1.

——— sulochrin
---------- compound G
—·—·— compound G dimethyl ether methyl ester

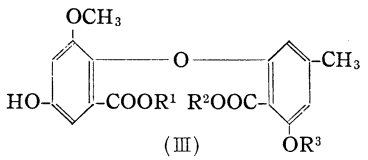
spiran derivative (II) and compounds B (sulochrin) and G are benzophenones (I, R^1:R^3:CH^3, R^2:R^4:H and R^1:R^2:R^4:H, R^3:CH_3). According to the proposed biogenetic sequence[8] from benzophenone (I) to diphenyl ether (III) through spiran (II) by oxidative coupling followed by hydrolysis, compound G could be assumed as the precursor of compound F, while sulochrin (compound B) is that of compound A.



(I)



(II)



(III)

Experimental*5

Compound G——Crude compound G,[3] isolated from mycelium of *Oospora sulphurea-ochracea* accompanied by compound F, was further purified by crystallization from AcOEt-light petroleum (b.p. 45~80°) or MeOH-water to afford pale yellow prisms, m.p. 205~207° (with effervescence). It dissolves in aq. $NaHCO_3$ as bright yellow solution and gives a purple-brown ferric chloride reaction. It is hygroscopic. *Anal.* Calcd. for $C_{16}H_{14}O_7 \cdot H_2O$: C, 57.14; H, 4.80; 1 COOH, 13.38; 1 CH_3O, 9.23. Found : C, 57.05; H, 4.69; COOH, 13.82; CH_3O, 8.82. IR ν_{max}^{Nujol} cm^{-1} : 3365 (OH), 1697 (COOH), 1634 (bonded Ar-CO-Ar), 1611, 1593 (phenyl), 1392, 1245, 1200, 1169, 1059, 914, 820. UV (cf. Fig. 1) λ_{max}^{EtOH} (log ε) : 282 (4.23).

Compound G Dimethyl Ether Methyl Ester——Compound G (20 mg.) in Me_2CO was treated with excess of CH_2N_2 in Et_2O for 2 days at room temperature. After evaporation the residue was recrystallized from MeOH to pale yellow prisms, m.p. 155~156°. The identity with sulochrin dimethyl ether[4] was proved by a mixed fusion and IR spectra. IR ν_{max}^{Nujol} cm^{-1} : 1719 ($COOCH_3$), 1630 (bonded Ar-CO-Ar), 1604 (phenyl), 1302, 1236, 1063, 842, 827. UV (cf. Fig. 1) λ_{max}^{EtOH} (log ε) : 285 (4.23).

Compound G Methyl Ester——Compound G (20 mg.) in anhyd. Et_2O was treated with CH_2N_2 in Et_2O for 1 min. under ice cooling and the excess of CH_2N_2 was decomposed with a drop of AcOH. After evaporation, the residue was recrystallized from MeOH-water to pale yellow needles of m.p. 253~256° (decomp.), which showed identity with sulochrin by a mixed fusion and IR spectra in Nujol.[1]

Decomposition of Compound G with Sulfuric Acid——Compound G (0.4 g.) was dissolved in H_2SO_4 (2.4 cc.) and, after 10 min., the deep red solution was poured into ice-water. The precipitate was taken in Et_2O which was treated with $NaHCO_3$ solution to remove acidic substances. Acidification of the $NaHCO_3$ layer with HCl afforded colorless precipitate. After drying, the precipitate was extracted with boilig benzene (10 cc.) and filtered. The insoluble portion was recrystallised from water to give colorless needles of m.p. 199~200°, which showed no depression of m.p. when fused

*5 M.p.s. were determined in a sulfuric acid bath and uncorrected. Infrared spectra were measured using a Koken Model 301 infrared spectorophotometer and ultraviolet spectra were determined for EtOH solution in a Carry Model II recording spectrophotometer.

with α-resorcillic acid monomethyl ether.[4]

From the mother liquor of the benzene extracts colorless needles separated out after cooling, which showed m.p. 158~162° (decomp.) on recrystallization from benzene. A mixed fusion with *p*-orsellinic acid (m.p. 171~172°) melted at 161~166° and the IR spectra of both specimens in Nujol were almost identical. Methylation of the acid with CH_2N_2 for a short time gave colorless needles from MeOH-water, m.p. 97~98°. A mixed fusion with methyl *p*-orsellinate showed no depression of m.p.

Treatment of Compound G with Methanolic Potassium Hydroxide——Compound G (0.2 g.) was refluxed with MeOH-KOH (10%, 10 cc.) for 30 min. Dilution with water and acidification with HCl afforded precipitate, which was collected and dried; yield, 0.15 g. It darkened around 280° and did not show any definite m.p. The properties were identical with those of 3-methyl-1,6-dihydroxy-8-xanthonecarboxylic acid.[4]

The sublimation under reduced pressure gave colorless product, m.p. over 300°, which was proved to be identical with 1,6-dihydroxy-3-methylxanthone[7] by IR spectrum.

We thank Professor S. Shibata, University of Tokyo, for his interest and helpful suggestion. We are indebted to Dr. E. Kimura and his coworkers for microanalyses, to Mrs. E. Tanaka and Miss N. Ninomiya for IR spectra and to Miss H. Nagata for UV spectra.

Summary

Compound G, a metabolite of *Oospora sulphurea-ochracea* v. Beyma, was shown to be desmethylsulochrin (I, R^1:R^2:R^4:H, R^3:CH_3).

(Received June 19, 1961)

UDC 615.771.7:547.852.2

Takanobu Itai and Shigeru Sako: Potential Anti-cancer Agents. IV.[1] 3-Substituted 6-Chloropyridazine 1-Oxides.

(*National Institute of Hygienic Sciences*[*1])

In Part III of this series, the N-oxidation of pyridazine derivatives was reported.[1] N. Takabayashi[2] oxidized 3-methoxy-6-chloropyridazine to its N-oxide, but the position of the N-oxide was not yet determined. H. Igeta[3] obtained 3-methoxypyridazine 1-oxide by oxidation of 3-methoxypyridazine, and M. Kumagai[4] published a paper on the N-oxidation of 3-methylpyridazine and its derivatives, bearing an ethoxy or a phenyl group at the 6-position of the molecule. The position of the oxide was not made clear in the former two cases, but it was shown to be the 2-position in the latter case. Although N. Takabayashi[2] and H. V. Euler *et al.*[5] failed to gain 3,6-dichloropyridazine N-oxide (II) from 3,6-dichloropyridazine (I) with hydrogen peroxide in glacial acetic acid, they obtained 6-chloro-3(2*H*)-pyridazinone due to hydrolysis. H. Igeta[6] obtained 3-chloropyridazine 1-oxide by oxidation of 3-chloropyridazine with perbenzoic acid.

*1 Tamagawa-yoga, Setagaya, Tokyo (板井孝信, 佐子 茂).

1) Part III. T. Itai, S. Sako: This Bulletin, **9**, 149 (1961).
2) N. Takabayashi: Yakugaku Zasshi, **76**, 1293 (1956).
3) H. Igeta: This Bulletin, **7**, 938 (1959).
4) M. Kumagai: Nippon Kagaku Zasshi, **81**, 1148 (1960).
5) H. V. Euler, H. Hasselquist, O. Heidenberger: C. A., **54**, 12156b (1960); Arkiv. Kemi., **14**, 419 (1959).
6) H. Igeta: This Bulletin, **8**, 559 (1960).