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Studies on the Metabolic Products of *Oospora astringenes*.[1] VIII.[2] Isolation, Chemical Structure, and Biosynthesis of Oospolide

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A new metabolic product of *Oospora astringenes*, oospolide, was isolated by ion-exchange chromatography of the culture medium, and the structure was determined as the formula (I). The biosynthesis of oospolide was also studied by using ^{14}C-acetates, and head-to-tail condensation of acetate units was presumed as the biosynthetic mechanism.

Some isocoumarin derivatives such as oospolactone, oosponol, and oospoglycol had been isolated from *Oospora astringenes* YAMAMOTO, and their chemical structures were determined. Biogenesis of these isocoumarins had also been studied by using ^{14}C-labeled compounds and was assumed that they are biosynthesized from one C_1-unit and one polyketomethylene chain which is formed from five C_2-units *via* acetate-malonate pathway. In the course of administration experiments with acetate [2-^{14}C], contrary to the case of formate-^{14}C, a new compound was detected as a radioactive spot on the paper chromatogram of the culture medium. This was also detectable in the "cold" experiment as a quenching spot under radiation of ultraviolet ray, and as a yellow spot when Bromphenol Blue or sodium hydroxide solution was sprayed. This paper deals with the isolation, chemical structure, and biosynthesis of the new compound.

This compound was so easily soluble in water that extraction with organic solvent was ineffective, and ion-exchange chromatography was used for isolation. Thus, the culture medium obtained by 30—40 days' cultivation was passed through a column of Dowex-1×8 (Cl^- type), and the adsorbed part was eluted with 0.05N hydrochloric acid. After concentration, all the effluent was rechromatographed on a column of Dowex-1×8 (formate type) by gradient elution using 0.0—0.3N hydrochloric acid. The effluent was divided into two fractions by optical density at 265 mμ. From the first fraction, the new compound was obtained as colorless prisms and was named oospolide (I). The second fraction was assumed as an artifact produced from oospolide by the action of hydrochloric acid.

Oospolide (I) was insoluble in petroleum benzin and benzene, slightly soluble in chloroform and ethyl acetate, and very soluble in ethanol, dioxane, and water. The empirical formula $C_8H_{10}O_5$ was determined for oospolide (I) by elementary analysis, titration with sodium hydroxide, and mass spectrometry (M^+ 186). It had strong acidity (pK_a' 3.5) and showed no optical activity. It was positive for enol test,[4] Legal reaction, Ehrlich reaction, and consumed periodate.

Oospolide (I) showed strong ultraviolet (UV) absorption at 260—264 mμ ($\lambda_{max}^{CHCl_3}$ 260 mμ, $\varepsilon=12000$; λ_{max}^{EtOH} 261 mμ, $\varepsilon=12600$; $\lambda_{max}^{H_2O}$ or $\lambda_{max}^{0.1-1N\,HCl}$ 264 mμ, $\varepsilon=12600$) which shifted to longer

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[1] A part of this work was presented at the 89th Annual Meeting of the Pharmaceutical Society of Japan, Nagoya, April 1969, and was reported in preliminary forms (K. Nitte, Y. Yamamoto and Y. Tsuda, *Tetrahedron Letters*, **1967**, 3013; *idem, ibid.*, **1968**, 4231).

[2] Part VII: K. Nitta, Y. Yamamoto, T. Inoue, and T. Hyodo, *Chem. Pharm. Bull.* (Tokyo), **14**, 363 (1966).

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[4] F. Feigl, "Spot Tests in Organic Analysis," 6th Ed., Maruzen Asian Ed., Maruzen Co., Tokyo, 1961, p. 213.

wave length by about 30 mμ on basification ($\lambda_{\text{max}}^{0.1-1\text{N NaOH}}$ 296 mμ, ε=14100). When the basified solution was re-acidified with hydrochloric acid, the acid solution no longer showed the original spectrum, but showed a new one (λ_{max} 267 mμ, ε=*ca.* 2000). This solution gave instant violet color with ferric chloride in contrast to oospolide which showed gradual coloration. Re-basification of this solution produced the spectrum identical with that of oospolide in alkaline solution. When oospolide was titrated with sodium hydroxide, it behaved as a monobasic acid, whereas it was titrated as a dibasic acid (p*K*a′ 3.5, 8.3) in inverse titration. The UV absorption of oospolide in alkaline solution gradually diminished and showed no absorption after 12 hr. These facts indicate very rapid transformation of oospolide into a β-diketone-type compound[5] in alkaline condition prior to complete decomposition.

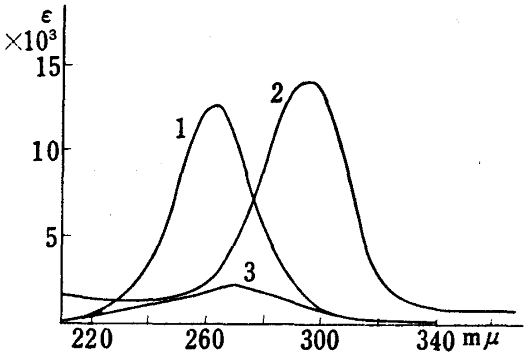

Fig. 1. UV Spectra of Oospolide (I)

1. in H_2O or 0.1—1N HCl
2. in 0.1—1N NaOH
3. in HCl after treatment with NaOH

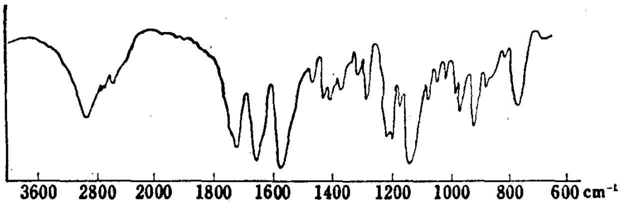

Fig. 2. IR Spectrum of Oospolide (I) (KBr)

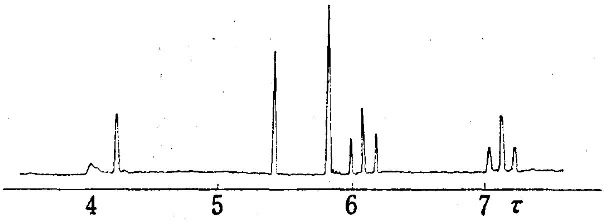

Fig. 3. NMR Spectrum of Oospolide (in $CDCl_3$)

The infrared (IR) spectrum of oospolide (I) showed absorption at 2800—2500 (broad, ν_{OH}, chelated), 1735 (C=O), 1660 (C=O), 1570 cm^{-1} (C=C), and so on.

Oospolide (I) showed nuclear magnetic resonance (NMR) signals (in $CDCl_3$) at τ 7.14, 6.12 (2H, triplet, *J*=6.0 cps), 5.85 (2H, singlet), 5.46 (2H, singlet, disappeared gradually on addition of D_2O), 4.29 (1H, singlet). These signals were assigned to $-CH_2CH_2-O-$, $-CH_2-O-$, $-O-CH_2-CO-$, and $-CH=$, respectively. Oospolide showed another signal at τ 4.0 (1H, broad singlet, disappeared on addition of D_2O), which was assigned to enolic OH or COOH group although its chemical shift seemed too high.

Oospolide (I) was smoothly methylated with diazomethane to an oily methylate (II), which showed IR absorptions at 1750 (C=O), 1680 (C=O), 1590 cm^{-1} (C=C), while the absorption at 2800—2500 cm^{-1} in oospolide (I) disappeared. In NMR spectrum (in $CDCl_3$), II had the signal due to OCH_3 group at τ 6.24 (3H, singlet), while the signal at τ 4.0 in oospolide disappeared. Oospolide also gave mono-*p*-bromophenacyl derivative (III), $C_{16}H_{15}O_5Br$, mp 112°.

On permanganate oxidation or ozonolysis, oospolide gave a dicarboxylic acid (IV), $C_5H_8O_5$, mp 94°, which presumed to be $HOOC-CH_2CH_2-O-CH_2-COOH$ by the analysis of NMR spectrum [in D_2O, τ 7.30, 6.16 (2H, triplet, *J*=6.0 cps), 5.79 (2H, singlet)]. This product was identified with the specimen obtained by permanganate oxidation of tetrahydro-γ-pyrone.[8] This acid showed pK_a' 3.6 and 4.7, the former agreed well with that of oospolide (I). When

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[5] Acetyl acetone, UV $\lambda_{\text{max}}^{H_2O}$ 274 mμ (ε=1500[6]); $\lambda_{\text{max}}^{1\text{N NaOH}}$ 294 mμ (ε=20000[6]); pK_a 8.13,[7] purple with $FeCl_3$.
[6] G.S. Hammond, W.G. Borduin, and G.A. Guter, *J. Am. Chem. Soc.*, **81**, 4682 (1959).
[7] G. Schwarzenbach and K. Lutz, *Helv. Chim. Acta*, **23**, 1147 (1940).
[8] W. Borshe and K. Thiele, *Chem. Ber.*, **56**, 2012 (1923).

the ozonide of oospolide was treated with hot water, glycolic acid was obtained accompanied with the acid (IV). On reductive degradation, the ozonide gave glycolaldehyde.

Catalytic hydrogenation of oospolide (I) over palladium–charcoal at *ca.* 50°, gave dihydro-oospolide (V), which had a strong acidity (pK_a' 3.6) like oospolide (I). On treatment with diazomethane, it gave an oily monomethyl derivative, $C_9H_{14}O_5$ (VI). Mono-*p*-bromophenacyl derivative, $C_{16}H_{17}O_6Br$ (VII), mp 88°, was also obtained by treatment with *p*-bromophenacyl bromide. IR spectrum of dihydro-oospolide (V) showed absorption at 2800—2500 cm^{-1} as well as oospolide, but in the carbonyl region, it showed a single strong absorption at 1750 cm^{-1}, while the absorption at 1570 cm^{-1} (C=C) in oospolide disappeared. NMR spectrum (in $CDCl_3$) of dihydro–oospolide (V) showed the signal at τ 3.2 (disappeared on addition of D_2O), and no signal of olefinic proton. These results confirmed the presence of a double bond in oospolide, and the acidity of oospolide was not due to enolic OH but to COOH group. Further, the pK_a' values of oospolide (I) and dihydro–oospolide (V) coincided with that of CH_3-O-CH_2-COOH (pK_a 3.35⁹⁾) rather than CH_3–O–CH_2CH_2–COOH (pK_a 4.46⁹⁾). These facts about p*K*a and the structure of IV suggested a partial structure of –CH_2CH_2–O–CH_2–COOH for oospolide (I).

Although oospolide did not give pure 2,4-dinitrophenylhydrazone, dihydro–oospolide methyl ester (VI) gave a crystalline 2,4-dinitrophenylhydrazone (VIII), $C_{15}H_{18}O_8N_4$, mp 115°, which still exhibited the absorption due to α-oxygenated $COOCH_3$ group at 1750 cm^{-1}. Since dihydro–oospolide methyl ester (VI) showed a single carbonyl absorption at 1750 cm^{-1}, the ketonic group in VI, which forms 2,4-dinitrophenylhydrazone, must have shown the absorption around 1750 cm^{-1} overlapping with the band of $COOCH_3$ group. Hence, the ketonic group must be present on a five membered ring.

The UV absorption of oospolide (I) disappeared on borohydride reduction or catalytic hydrogenation, and the IR absorption of ketonic group of oospolide shifted to a higher wave number on hydrogenation (1660→1750 cm^{-1}). Therefore, the chromophoric group of oospolide which corresponded to IR absorptions at 1660 and 1570 cm^{-1} was attributed to a conjugated ketone. In addition to these results, oospolide had an NMR signal at τ 5.46 which was assigned to –CO–CH_2–O– grouping. From these results, oospolide (I) was presumed to have the partial structure shown in Fig. 4. Oospolide was formerly¹⁾ presumed to be degraded to a β-diketone-type compound by the action of alkali and this may be interpreted as a hydrolytic cleavage of the enol ring.

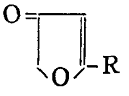


Fig. 4

Further, the NMR and NMDR spectra of dihydro–oospolide *p*-bromophenacyl ester (VII) in deuterochloroform gave many informations on the structure of oospolide. VII showed signals at τ 2.28, 4.63 (each due to *p*-bromophenacyl group), 5.72 (singlet, **a**), 6.22 (triplet, **b**), 7.95 (quartet, **c**), 5.50 (multiplet, **d**), 7.54 (multiplet, **e**), and 6.06 (AB-quartet, **f**). The signals **a**, **b**, **c**, and **f** correspond to those at τ 5.85, 6.12, 7.14, and 5.46 in oospolide, respectively.

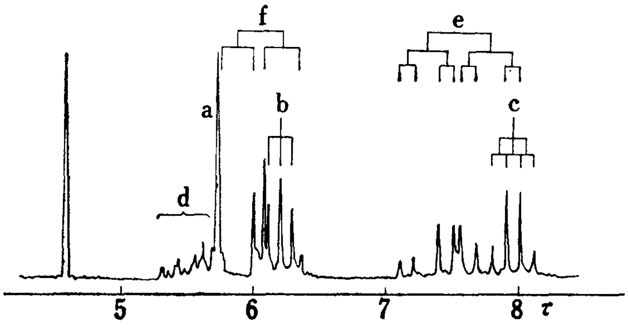
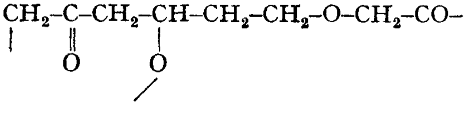


Fig. 5. NMR Spectrum of Dihydro-oospolide *p*-Bromophenacyl Ester (VII) (in $CDCl_3$)



$$\underset{|}{\overset{\text{f}}{\mathrm{CH_2}}}\text{–}\underset{\underset{\mathrm{O}}{\|}}{\mathrm{C}}\text{–}\overset{\text{e}}{\mathrm{CH_2}}\text{–}\underset{\underset{\mathrm{O}}{|}}{\overset{\text{d}}{\mathrm{CH}}}\text{–}\overset{\text{c}}{\mathrm{CH_2}}\text{–}\overset{\text{b}}{\mathrm{CH_2}}\text{–O–}\overset{\text{a}}{\mathrm{CH_2}}\text{–CO–}$$

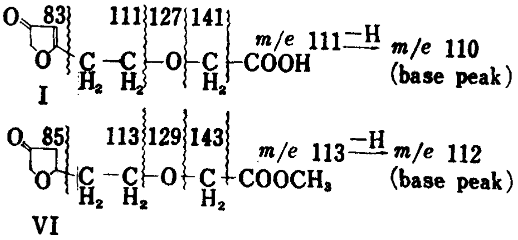


Fig. 6

The signal at τ 7.14 (triplet, CH_2) in oospolide (I) showed a remarkably high magnetic field shift and became a quartet by hydrogenation. Hence, the double bond in oospolide was adjacent to $-CH_2CH_2-O-$ grouping. On irradiation of **b**, the signal **c** turned to a doublet. Irradiation of **c** changed **b** to a singlet, and simplified the signal **d**. When **d** was irradiated, **c** changed to a triplet and **e** to an AB-quartet.

From all the foregoing experimental evidences, the structure (I) was assigned to oospolide. This structure was also supported by mass spectra as shown in Fig. 6.

The structure (I) was finally confirmed by the synthesis of deoxodihydro-oospolide (IX) which was derived from dihydro-oospolide (V) by the following way. V was treated with ethanethiol in the presence of zinc chloride and sodium sulfate, and the thioketal obtained was desulfurized with Raney nickel to an oily deoxodihydro-oospolide (IX), which was crystallized as its *p*-bromophenacyl ester (X), $C_{16}H_{19}O_5Br$, mp 75°. The synthesis of this compound was carried out by condensation of ethyl bromoacetate to sodio derivative of 2-(tetrahydro-2-furyl)-ethanol[10] (XI), and the product was hydrolyzed with sodium hydroxide to the corresponding acid. The *p*-bromophenacyl ester of this acid was found to be identical with the specimen obtained from the natural product. All the reactions of oospolide are summarized in Chart 1.

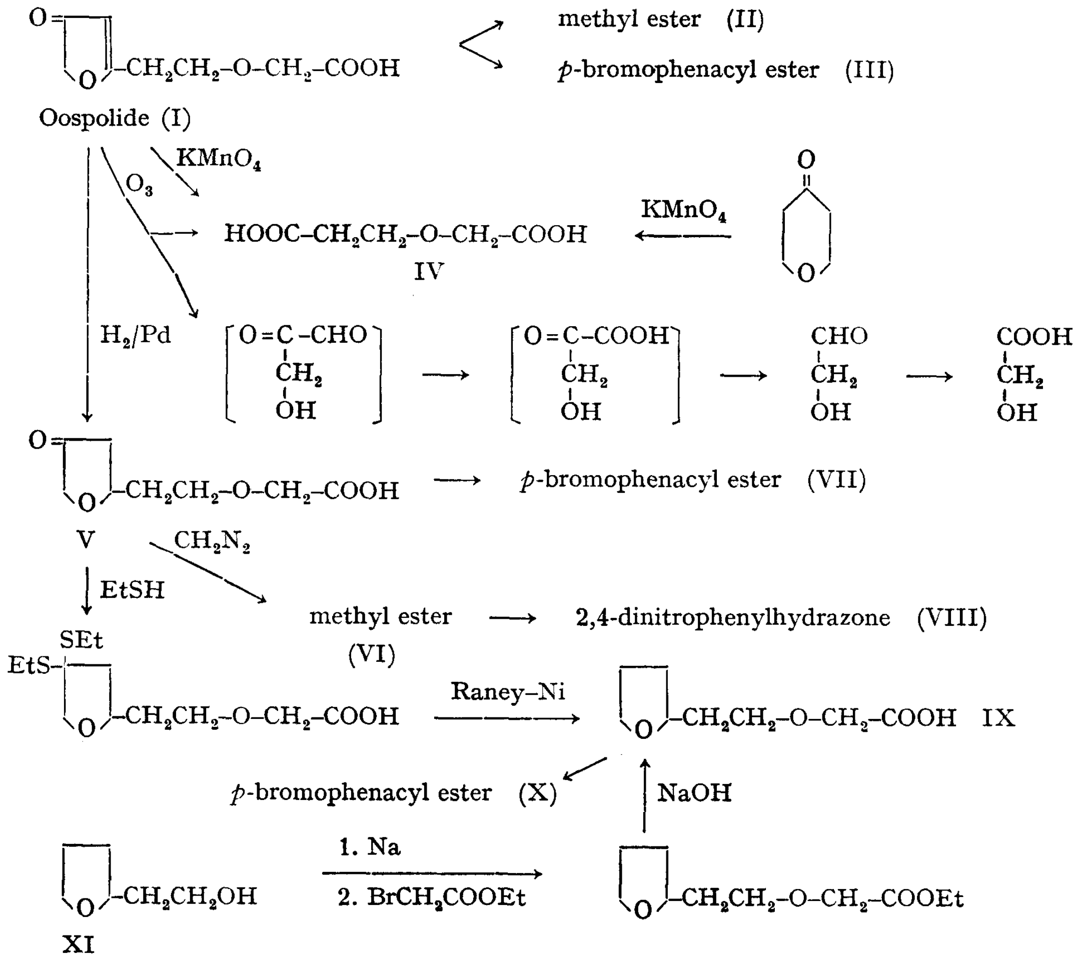


Chart 1

The biosynthesis of oospolide (I) was studied by using acetate[1-^{14}C] and [2-^{14}C]. The labeled compounds were administered to the culture of *Oospora astringenes* at the middle period of cultivation, and harvested about 2—3 weeks later. Labeled oospolide was isolated

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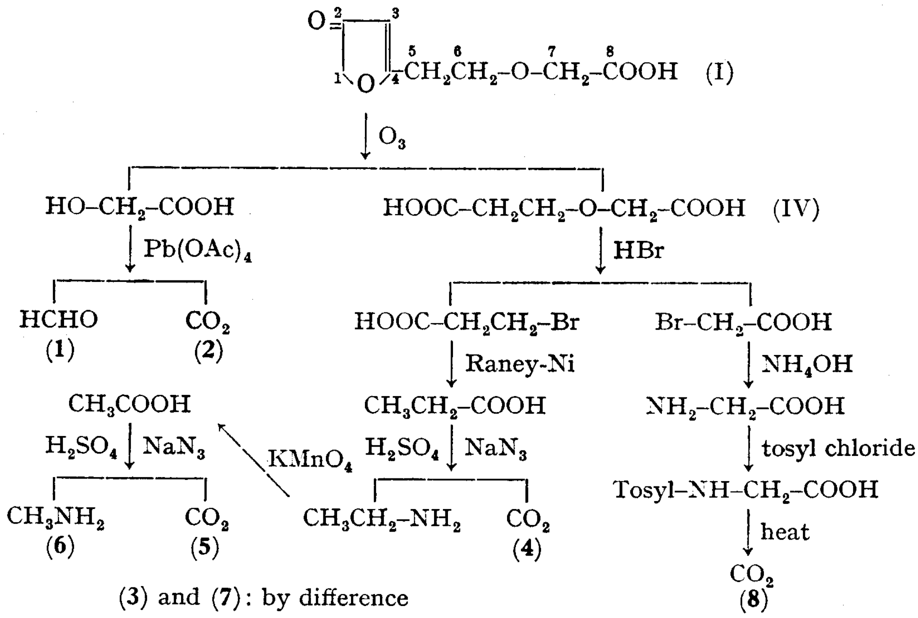
9) "Jikkenkagaku-Koza," Supplement Vol. 5, Maruzen Co., Tokyo, 1966, p. 732.
10) P.M. Frearson, D.G. Hardy, and E.S. Stern, *J. Chem. Soc.*, **1960**, 2103.

by ion-exchange chromatography as described above. Incorporation of radioactivity into oospolide was 1.17 and 0.41% in the experiments with acetate[2-^{14}C] and acetate[1-^{14}C], respectively. Radioactive oospolide was degraded by the procedure shown in Chart 2, and the distribution of radioactivity was determined. The results are summarized in Table I.



(3) and (7): by difference

Chart 2. Degradation of Oospolide

Number in ( ) shows the carbon number of oospolide.

TABLE I. Percentage Distribution of Radioactivity in Oospolide

Carbon No.[a]	Experiment with	
	CH$_3$^{14}COONa (%)	^{14}CH$_3$COONa (%)
1	0.6	23.1
2	18.7	2.2
3	2.0[b]	21.0[b]
4	23.2	28.4
5	4.7	
6	25.4	
7	8.9[b]	25.3[b]
8	16.3	

a) The carbon number on oospolide is presented in Chart 2.
b) These values were obtained by difference.

In the experiment with acetate[2-^{14}C], higher radioactivity was found in carbons 2, 4, 6, and 8, and lower activity in carbons 1, 3, 5, and 7. In the experiment with acetate[1-^{14}C], high and low radioactivities were distributed alternately in the molecule of oospolide, though full data could not be obtained owing to poor radioactivity. These results showed that carbons 1, 3, 5, and 7 were derived from the methyl-carbon of acetate, and 2, 4, 6, and 8 from the carboxyl-carbon; namely, oospolide was biosynthesized by head-to-tail condensation of four acetate units.

Experimental

Cultivation of *Oospora astringenes*——The culture medium consisted of malt extract, 20 g; anhydrous glucose, 20 g; peptone, 1 g, made to 1 liter with tap water. Cultivation was carried out by the method described in Part VII[2] of this work.

Isolation of Oospolide (I)——Culture medium (3 liters) obtained by 30—40 days' cultivation was passed through a column (3×50 cm) of Dowex-1×8 (Cl^- type). After the column was washed with H_2O, the adsorbed part was eluted with 3 liters of 0.05N HCl. The yellow eluate was concentrated *in vacuo* below 40° to about 50 ml and re-adsorbed on a column (3×50 cm) of Dowex-1×8 (formate type). After washing with H_2O, it was fractionated by gradient elution using 0.0—0.3N HCl (volume, 4 liters). The effluent was divided into two fractions by optical density at 265 mμ. The first fraction (*ca.* 2 liters, slightly yellow) was evaporated to dryness in vacuum below 40° (all HCl was carefully eliminated by repeated addition and evaporation of H_2O). The syrupy brown residue was crystallized from EtOH, and recrystallized from the same solvent to colorless prisms, mp 111°; yield, 50—100 mg from 3 liters of culture. *Anal.* Calcd. for $C_8H_{10}O_5$: C, 51.61; H, 5.41. Found: C, 51.58; H, 5.48. Titration equivalent, Calcd.: 186. Found: 186. Mass Spectrum *m/e*, 187 (0.7, $M^+ + 1$), 186 (0.6, M^+), 141 (2.2), 127 (3.3), 111 (19), 110 (100), 98 (22), 83 (12), etc.

***p*-Bromophenacyl Ester (III)**——To a solution of oospolide (I) (60 mg) in H_2O (1 ml) (adjusted to pH 5 with NaOH), *p*-bromophenacyl bromide (90 mg) in EtOH (5 ml) was added and boiled for 1.5 hr. When cooled, H_2O was added and the resulting precipitate (80 mg) was recrystallized from EtOH as leaflets (III), mp 112°. *Anal.* Calcd. for $C_{16}H_{15}O_6Br$: C, 50.15; H, 3.95. Found: C, 49.84; H, 4.00.

Permanganate Oxidation——To a solution of oospolide (I) (270 mg) in H_2O (10 ml), 2% $KMnO_4$ (50 ml) was added gradually at room temperature. The resulting MnO_2 was filtered off, the filtrate was acidified with H_2SO_4, and extracted continuously with ether for 30 hr. The solvent was evaporated from the extract and the residual colorless crystals were recrystallized from iso-Pr_2O as needles (IV), mp 94° (yield, 67 mg). *Anal.* Calcd. for $C_5H_8O_5$: C, 40.54; H, 5.44. Found: C, 40.27; H, 5.30. This compound was identified with β-carboxymethoxypropionic acid obtained by $KMnO_4$ oxidation of tetrahydro-γ-pyrone.

Ozonolysis of Oospolide——Oospolide (I) (300 mg) was suspended in $CHCl_3$ (30 ml) and treated with ozone for 1 hr under ice-cooling. After removal of the solvent, H_2O was added to the residue and steam-distilled. In the distillate neither acid nor aldehyde was detected. Non-volatile part was extracted continuously with ether for 40 hr. The solvent was evaporated and the resulting crystals were collected (50 mg), which was identified with IV. The syrupy mother liquor was paper-chromatographed (Toyo Roshi No. 50, 40×40 cm, 3 sheets. Solvent: EtOH: conc. $NH_4OH:H_2O=160:10:30$). The band at *Rf* value of about 0.5 (positive to the Nessler reagent) was cut out, sprayed with dil. HCl, and extracted with EtOH. After removal of the solvent, the residue was neutralized with KOH and evaporated to dryness (yield, 50 mg). The resulting colorless powder was treated with S-benzylpseudothiuronium chloride by the usual method. The S-benzylpseudothiuronium salt, mp 140°, was identified with that of glycolic acid by IR spectrum and mixed melting point.

When the ozonide (from 300 mg of oospolide) was steam-distilled after treatment with aqueous SO_2 solution, the distillate gave 2,4-dinitrophenylhydrazone. The precipitate was recrystallized from EtOH to orange needles, mp 150° (decomp.), which was identified with glycolaldehyde 2,4-dinitrophenylhydrazone. From the non-volatile fraction, IV and glycolic acid were also obtained.

Catalytic Hydrogenation of Oospolide——Oospolide (200 mg) in EtOH (10 ml) was hydrogenated over 400 mg of 5% Pd-C with heating to about 50° by an IR lamp. After *ca.* 26 ml of hydrogen was absorbed, the catalyst was filtered off and the solvent was evaporated. The residual syrup was treated with ether solution of CH_2N_2. The methyl ester (VI) obtained was purified by vacuum distillation (1 mmHg, bath temperature, 170°). *Anal.* Calcd. for $C_9H_{14}O_5$: C, 53.46; H. 6.98. Found: C, 53.18; H, 7.20. Mass Spectrum *m/e*, 202 (10, M^+), 143 (27), 129 (16), 114 (25), 113 (29), 112(100), 103 (38), 94 (31), 91 (27), 85 (61), 84 (46), etc.

p-Bromophenacyl ester (VII) was prepared by the usual method as colorless leaflets, mp 88°. *Anal.* Calcd. for $C_{16}H_{17}O_6Br$: C, 49.89; H, 4.55. Found: C, 49.86; H, 4.55.

Dihydro-oospolide methyl ester (VI) (150 mg) was treated with 2,4-dinitrophenylhydrazine solution [100 mg in conc. H_2SO_4 (0.5 ml), H_2O (0.8 ml), and EtOH (25 ml)] and stood overnight. The resulting precipitate was recrystallized from EtOH as orange needles (VIII), mp 115°. *Anal.* Calcd. for $C_{15}H_{18}O_8N_4$: C, 47.12; H, 4.75; N, 14.66. Found: C, 47.40; H, 4.87; N, 14.40.

By the same method, dihydro-oospolide (V) gave 2,4-dinitrophenylhydrazone ethyl ester, mp 101°. *Anal.* Calcd. for $C_{16}H_{20}O_8N_4$: C, 48.48; H, 5.09; N, 14.14; OC_2H_5, 11.35. Found: C, 48.28; H, 5.10; N, 13.86; OC_2H_5, 11.2. NMR ($CDCl_3$) τ: 8.72 (3H, triplet, $J=7.1$ cps, CH_3), 5.77 (2H, quartet, $J=7.1$ cps, O–CH_2–Me).

Deoxodihydro-oospolide (IX)——Dihydro-oospolide (670 mg) was added to a mixture of EtSH (4 ml), anhyd. $ZnCl_2$ (0.3 g), and anhyd. Na_2SO_4 (0.2 g). After standing for 20 hr at 4° followed by occasional shaking at room temperature for 6 hr, 5 ml of H_2O was added and EtSH was evaporated at room temperature. Extraction with ether and evaporation gave a syrupy thioketal. This was dissolved in 100 ml of 70% EtOH without purification, and boiled for 5 hr with Raney Ni (prepared from 25 g of 50% Raney alloy). When cooled, Raney Ni was collected by centrifugation and washed with EtOH. As the product was adsorbed on the Raney Ni, it was dissolved in 10% HCl (*ca.* 100 ml) and the resulting greenish solution was extracted with ether. The ether extract was treated with 10% $NaHCO_3$ and the aqueous layer was acidified and re-extracted with ether. By evaporation of the solvent, deoxodihydro-oospolide (IX) was obtained as cololoress syrup (yield, 300 mg). It was treated with *p*-bromophenacyl bromide by the usual method, and *p*-bromophenacyl ester thereby obtained was recrystallized from iso-Pr_2O as colorless leaflets (X), mp 75°. *Anal.* Calcd. for $C_{16}H_{19}O_5Br$: C, 51.77; H, 5.16. Found: C, 51.46; H, 5.17. From the supernatant of Raney Ni treatment, ethyl ester of IX was obtained as colorless oil (yield, 200 mg).

Synthesis of Deoxodihydro-oospolide (IX) and Its *p*-Bromophenacyl Ester——In 4 g of 2-(tetrahydro-2-furyl)ethanol (XI), which was prepared from tetrahydrofurfuryl bromide by the method of Frearson, *et al.*,[10] 0.2 g of Na metal was dissolved by warming. Ethyl bromoacetate (1.5 g) was added to this solution and heated on a boiling water bath for 2 hr. When cooled, H_2O was added to this mixture and extracted with ether. The solvent was distilled off and the residue was fractionated by vacuum distillation. The fraction distilling at 136—140° at 2 mmHg (1.5 g) was suspended in 30 ml of 1N NaOH and heated on a boiling water bath for 3 hr. The cooled reaction mixture was acidified and extracted with ether. On evaporation of the solvent a syrupy acid substance (identical with IX) was obtained. It was crystallized as *p*-bromophenacyl ester and identified with the ester obtained from the natural product by IR, UV, and NMR spectra, and mixed melting point.

Tracer Experiments with ^{14}C-labeled Compounds——Each tracer experiment was carried out using four 3-liters Fernbach flasks containing 800 ml of the malt extract medium by the method described in Part VII[2] of this series. $CH_3{}^{14}COONa$ (*ca.* 1 mCi, 1000×10^6 cpm) was added on the 17 th day of cultivation, and the culture was harvested on the 37th day. Yield of labeled oospolide, 101 mg, 41.0×10^5 cpm. In another experiment, $^{14}CH_3COONa$ (*ca.* 0.1 mCi, 101×10^6 cpm) was added on the 18th day, and harvested onthe40th day. Yield of labeled oospolide, 120 mg, 12.4×10^5 cpm. The labeled oospolide was diluted with non-labeled sample and degraded for the determination of the distribution of radioactivity. Radioactivity was determined by a gas-flow counter (Kobe Kogyo, PR-123).

Degradation of Oospolide (I) for Determination of Radioactivity Distribution——In the tracer experiments, the labeled oospolide was degraded by the procedure summarized in Chart 2. Diluted labeled oospolide (*ca.* 500 mg) was degraded by ozonolysis into glycolic acid and β-carboxymethoxypropionic acid (IV), and the former was further degraded with $Pb(OAc)_4$ by the described method[11] into HCHO (carbon 1) and CO_2 (carbon 2), which were converted to 2,4-dinitrophenylhydrazone and $BaCO_3$, respectively. β-Carboxymethoxypropionic acid (IV) (diluted with non-labeled sample to 1 g) was boiled with 20 ml of 48% HBr for 24 hr, diluted with H_2O, and extracted with ether. The solvent was evaporated, β-bromopropionic acid crystallized from the syrupy residue was collected (380 mg), and recrystallized from CCl_4. The syrupy mother liquor containing bromoacetic acid was dissolved in 50 ml of 28% NH_4OH. After standing for 48 hr at room temperature, NH_4OH was distilled off under reduced pressure, and the residue was treated with *p*-toluenesulfonyl chloride by the usual method. The resulting tosyl glycine (carbons 7 and 8) was purified by several recrystallization from AcOEt-petr. benzin, and decarboxylated (carbon 8) by the method of Cavalieri, *et al.*[12] The purified β-bromopropionic acid (300 mg) was dissolved in H_2O and heated with Raney Ni (prepared from 10 g of 50% Raney alloy) on a boiling water bath for 12 hr. The resulting propionic acid (carbons 4, 5, and 6) was steam-distilled and isolated as its sodium salt (200 mg). An aliquot of the salt (20 mg) was converted into *p*-bromophenacyl ester. Another part of the propionate was degraded by the Schmidt reaction into $EtNH_2$ (carbons 5 and 6) and CO_2 (carbon 4), and the latter was precipitated as $BaCO_3$. $EtNH_2$, which was collected as its hydrochloride, was oxidized with $KMnO_4$ into AcOH and further degraded by the Schmidt reaction into $MeNH_2$ (carbon 6) and CO_2 (carbon 5), which were collected as its picrate and $BaCO_3$, respectively. Radioactivities of all the solid products were determined, and the percentage distribution of radioactivity was calculated. The radioactivities of carbons 3 and 7, which could not be determined by direct counting, were calibrated by difference.

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