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21. Shinsaku Natori*1 and Hidejiro Nishikawa*2 : Structures of Osoic Acids and Related Compounds, Metabolites of *Oospora sulphurea-ochracea* v. Beyma.

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Seven metabolites, tentatively named compounds A to G, were isolated from *Oospora sulphurea-ochracea* v. Beyma in 1936~1937 by one of the authors (H.N.).[1,2] The major metabolite, compound B, which was later designated as sulochrin, was determined as being a benzophenone derivative (I).[3~5] The relationship of other members of the metabolites, compound A (V), m.p. 214°, compound D (II), m.p. 190°, and compound F (III), m.p. 248° (decomp.), was studied[6] and they were established as the monomethyl ether monomethyl ester, monomethyl ether dimethyl ester, and monomethyl ether, respectively, of the original acid (IV), named osoic acid, $C_{13}H_7O(OH)_3(COOH)_2$. Consequently, compound A was designated as dimethylosoic acid-I, compound D as trimethylosoic acid-I, and compound F as monomethylosoic acid. Methylation of the remaining two hydroxyl groups in osoic acids proceeded stepwise and several derivatives (VI~X) were prepared. On treatment with sulfuric acid, the osoic acids furnished the corresponding anhydro derivatives (XI~XIV), whose general molecular formula was represented as $C_{13}H_6O(OR)_3(CO)(COOR')$(R and R'=H or CH_3). Without any decisive evidence, Nishikawa[6] gave a conclusion that osoic acids (II~X) might be diphenyl ether derivatives and anhydro compounds (XI~XIV) might be xanthones.

Recent advances of chemistry of mold benzophenones and spiro-coumarans[7~20] prompted examination of the *Oospora* metabolites in the relation of those compounds. In regards to the molecular formula, the presence of one C-methyl group in osoic acid was suggested, which was actually proved by the Kuhn-Roth estimation. The ultraviolet spectra of osoic acid derivatives (II~X) were consistent with hydroxyphenoxybenzoic acid structures[21] and those of anhydro compounds (XI~XIV) were explained with hydroxy-

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19) D.H.R. Barton, T. Cohen : Festschrift A. Stoll, 117 (1957). Birkhäuser AG, Basel.
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TABLE I. Ultraviolet Spectra of Osoic Acid and Anhydro-osoic Acid Derivatives (in $10^{-4}\sim2.5\times10^{-5}M$ EtOH solution)

	λ_{max}^{EtOH} mμ(log ε)				
Trimethylosoic acid-I (compound D)(II=XV, $R^1=R^2=R^4=CH_3$, $R^3=R^5=H$)	213(4.65)	252(4.25)		312(4.01)	
Monomethylosoic acid (compound F)(III=XV, $R^4=CH_3$, $R^1=R^2=R^3=R^5=H$)	212(4.77)	251(4.22)		313(3.99)	
Osoic acid (IV=XV, $R^1=R^2=R^3=R^4=R^5=H$)	212(4.69)	251(4.24)		314(3.97)	
Dimethylosoic acid-I (compound A)(V=XV, $R^1=R^4=CH_3$, $R^2=R^3=R^5=H$)	213(4.70)	249(4.26)		316(4.02)	
Tetramethylosoic acid-I (VI=XV, $R^1=R^2=R^3=R^4=CH_3$, $R^5=H$)	211(4.67)	248(4.19)		315(3.96)	
Dimethylosoic acid-II (VII=XV, $R^3=R^4=CH_3$, $R^1=R^2=R^5=H$)	212(4.73)	251(4.13)		312(3.91)	
Pentamethylosoic acid (VIII=XV, $R^1=R^2=R^3=R^4=R^5=CH_3$)	208(4.86)		inf. 283(3.72)	307(3.77)	
Trimethylosoic acid-II (IX=XV, $R^3=R^4=R^5=CH_3$, $R^1=R^2=H$)	209(4.90)			298(3.81)	
Tetramethylosoic acid-II (X=XV, R^1 or $R^2=CH_3$, R^2 or $R^1=H$, $R^3=R^4=R^5=CH_3$)	208(5.00)		inf. 283(3.84)	307(3.94)	
Geodin hydrate (XVII)	216(4.71)			320(3.90)	
p-Orsellinic acid	213(4.50)	249(4.00)		306(3.56)	
Methyl *p*-orsellate	219(4.32)	257(4.11)		321(3.50)	
Methyl *p*-orsellate monomethyl ether	213(4.46)	258(4.10)		312(3.57)	
Anhydro-osoic acid (XI=XIX, $R^2=R^3=R^4=R^5=H$)	221(4.43)	263(4.49)		313(4.09)	348(3.75)
Tetramethylanhydro-osoic acid (XII=XIX, $R^2=R^3=R^4=R^5=CH_3$)	228(4.38)	254(4.54)	inf. 283(4.20)	306(4.13)	357(3.73)
Monomethylanhydro-osoic acid (XIII=XIX, $R^4=CH_3$, $R^2=R^3=R^5=H$)	223	263		312	347
Trimethylanhydro-osoic acid (XIV=XIX, $R^3=R^4=R^5=CH_3$, $R^2=H$)	228(4.45)	257(4.69)	inf. 284(4.23)	307(4.29)	358(3.82)

TABLE II. Infrared Spectra of Osoic Acid and Anhydro-osoic Acid Derivatives

Compound	State	1800∼1570 cm^{-1} region						900∼700 cm^{-1} region		
Trimethylosoic acid-I (Compound D)(II)	Nujol		1706	1658		1625	1603	1579	851	813
	$CHCl_3$	1724		1667		1629	1614	1584	825	
Monomethylosoic acid (Compound F)(III)	Nujol			1666(broad)		1630	1603		828	
Osoic acid (IV)	〃			1663(broad)		1630	1603	1582	857	833 728
Dimethylosoic acid-I (Compound A)(V)	〃	1714		1669		1625	1607	1589	828	
Tetramethylosoic acid-I (VI)	〃	1729	1707	1657			1603	1588	820	
	$CHCl_3$	1729		1665			1608	1586	825	
Dimethylosoic acid-II (VII)	Nujol		1718	1667		1632	1599	1579	825	
Pentamethylosoic acid (VIII)	〃	1737	1710				1608	1589	834	824
	$CHCl_3$	1729					1605(broad)		844	817
Trimethylosoic acid-II (IX)	Nujol		1707	1667			1603	1584	828	
	$CHCl_3$	1725	1695							
Tetramethylosoic acid-II (X)	Nujol	1738	1703				1604(broad)		846	
Geodin hydrate (XVII)	〃		1696	1660			1607		823	778(C-Cl)
Anhydro-osoic acid (XI)	〃			1659	1642		1608	1577		
Tetramethylanhydro-osoic acid (XII)	〃	1724			1638	1610	1597	1573		
Monomethylanhydro-osoic acid (XIII)	〃			1679	1641		1610	1578		
Trimethylanhydro-osoic acid (XIV)	〃	1758	1742		1622		1600	1572		
assignment		non-bonded COOR		bonded COOR	xanthone C=O		phenyl		C-H out of plane vending	

xanthone structure[22] (Table I). Infrared spectra also agreed with this assumption (Table II). Consequently, compounds A, D, and F would be represented as diphenyl ethers with two carboxyl (or methoxycarbonyl), one methoxyl, two hydroxyl, and one C-methyl groups.

The following facts would provide noticeable evidences for the arrangement of these substituents in the diphenyl ether molecules.

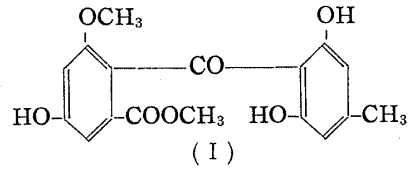
(a) Xanthone formation on dehydration reaction can only be explained by the presence of one carboxyl group at 2-position and no substituent at 6′-position.

(b) Presence of one each of hydrogen-bonded and non-bonded carboxyl (or methoxycarbonyl) group was indicated by the infrared spectra of compounds (II) to (VII), and the complete methylation of hydroxyl groups of these compounds (VIII～X) resulted in disapperance of the bonded carboxyl (or methoxycarbonyl) band (Table II). In the ultraviolet spectra, the methylation of the hydroxyl groups resulted in a shift of B-band about −10 mμ accompanying the disappearance of a band around 250 mμ (Table I). Since the infrared spectra of anhydro-osoic acid (XI) and monomethylanhydro-osoic acid (XIII) showed that the bonded carboxyl group is still retained, the non-bonded carboxyl in the parent diphenyl ethers must participate in the xanthone ring formation. All the hydroxyl groups of anhydro-osoic acid were completely methylated with diazomethane, which excludes the possibility of the presence of hydroxyls in 1- and 8-positions of the xanthone ring. This would suggest the absence of hydroxyls in 3- and 5′-positions of the original diphenyl ethers.

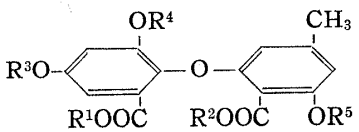
(c) Two of the three hydroxyls in osoic acid (IV) would not be in *ortho*- or *para*-position to each other, as it gave negative reaction with tetraamminecobalt(III) chloride and ammonium molybdate. Positive Gibbs reactions of osoic acid derivatives (II～VII) showed the absence of substituent at *para*-position to the bonded hydroxyl group.

(d) CH out-of-plane bending vibrations in infrared spectra of osoic acid derivatives (II～X) appeared in 820～830 cm^{-1} region. Although they were consistent with those of two adjacent C-H in benzene ring, some compounds having 1,2,3,5-tetrasubstituted benzene ring, such as *p*-orsellinic acid and some other lichen depsides, also show the band in the same region.[23] The ultraviolet absorption of osoic acid derivatives resembled those of *p*-orsellinic acid and geodin hydrate.[11,13]

Only two structures, (XV) and (XVI), could satisfy the above chemical and spectral results and are compatible with biogenetical relation. Of these two structures, the formula (XV) is more likely as it possesses all the substituents at the same position as those in sulochrin (I).



(I)



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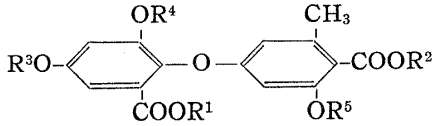


(XVI)

In 1959, Curtis, Hassall, and Jones[20] reported preliminarily the isolation of a diphenyl ether, asterric acid, from *Aspergillus terreus* Thom, giving a structure (XV : $R^1=R^4=CH_3$; $R^2=R^3=R^5=H$). Compound A and asterric acid might be identical. Since any details of

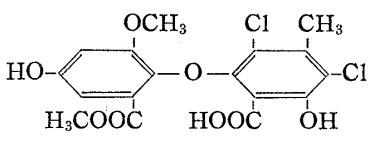
22) P. Yates, G.H. Stout : J. Am. Chem. Soc., 80, 1691 (1958).
23) Unpublish data in Professor S. Shibata's laboratory.

the properties, even m.p.s, of asterric acid and derivatives were not given at that time, experiment were made independently to elucidate the structure.

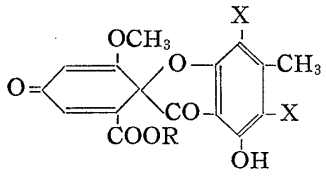
If the compound A has the same structure as asterric acid, it would be bisdichloro derivative of geodin hydrate[10,13] (XVII), which was obtained on hydrolysis of geodin[7~11] (XVIII: X=Cl; R=CH_3), another metabolite of *Aspergillus terreus*. For the purpose of establishing the correlation of osoic acids with geodin hydrate, chlorination of compound A (V) and pentamethylosoic acid (VIII) (a completely methylated derivative of compounds A, D, and F) was attempted. Treatment of compound A with molecular chlorine in chloroform solution afforded a trichloro derivative, m.p. 208~209°, irrespective of the amount of chlorine used. By treatment with sulfuryl chloride, compound A yielded a dichloro compound of m.p. 221~223° (decomp.) and a trichloro compound of m.p. 208~209°, depending on the amount of the reagent used. Geodin hydrate did not give a single reaction product on chlorination with sulfuryl chloride in chloroform, but it formed a single product, m.p. 207~209°, when a small amount of ethanol was added to the reaction mixture. The dichloro derivative of compound A was not identical with geodin hydrate, whereas the trichloro derivative was proved to be identical with the chloro derivative of geodin hydrate by infrared spectra and a mixed fusion. In the same way, pentamethylosoic acid gave dichloro, m.p. 187~189°, and a trichloro derivative, m.p. 146~150/164~166°, the latter was proved to be identical with monochlorinated product of geodin hydrate dimethyl ether methyl ester.[10,13]

From these facts, structure of dimethylosoic acid-I (compound A) was established as bisdechlorogeodin hydrate (XV: $R^1=R^4=CH_3$; $R^2=R^3=R^5=H$) and, thence, trimethylosoic acid-I (compound D) and monomethylosoic acid (compound F) would respectively be represented by formulae (XV: $R^1=R^2=R^4=CH_3$; $R^3=R^5=H$) and (XV: $R^4=CH_3$; $R^1=R^2=R^3=R^5=H$). Accordingly, anhydro-osoic acid derivatives should be expressed by (XIX). Treatment of monomethylanhydro-osoic acid (XIII; XIX, $R^4=CH_3$; $R^2=R^3=R^5=H$) with hydriodic acid gave a xanthone, which showed identical properties with norgeodin B[10] and should be 1-methyl-3,5,7-trihydroxyxanthone.

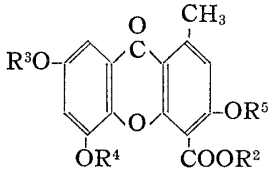
After completion of these experiments, the results were reported to Professor Hassall, University College of Swansea, to confirm their work on asterric acid. He kindly replied immediately, informing that they had just published their work on asterric acid in detail[24] and sending the samples of asterric acid derivatives. Asterric acid, methyl asterrate, and demethylasterric acid showed respective identities with the compounds A, D, and F by mixed fusion and infrared spectra. Hassall and his coworkers had established the structure of asterric acid chiefly by the formation of norgeodin-A (3-methyl-



(XVII)



(XVIII)



(XIX)

1,5,7-trihydroxyxanthone) and norgeodin-B (1-methyl-3,5,7-trihydroxyxanthone) and by its synthesis from sulochrin (I) by oxidative coupling with ferricyanide, followed by acid hydrolysis.

Another metabolite of *Oospora sulphurea-ochracea*, compound C, had m.p. 200° (decomp.), and a molecular formula, $C_{17}H_{16}O_8$, was reported in a previous paper.[1] Its ultraviolet spectrum revealed that the compound C might be a diphenyl ether derivative. Its infrared spectrum in Nujol mull showed absorptions at 1761, 1723, and 1692 cm^{-1},

24) R.F. Curtis, C.H. Hassall, D.W. Jones, T.W. Williams: J. Chem. Soc., **1960**, 4838.

which were respectively assigned to C=O stretchings in OCOR, non-bonded COOR, and non-bonded COOH. Analytical values reported in the previous paper[1] also agree with a molecular formula of $C_{19}H_{18}O_9$, and the presence of one acetyl, one carboxy, and two methoxyl groups was estimated. These facts indicated that the compound was methyl hydrogen acetoxyhydroxymethoxymethyldiphenyl ether-dicarboxylate.

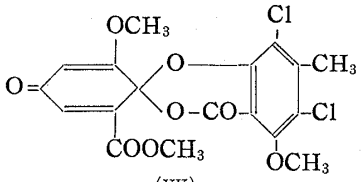
Hydrolysis of compound C with methanolic potassium hydroxide afforded monomethylosoic acid (compound F). Acetylation of compound C gave an acetate, which showed identity with diacetyldimethylosoic acid-I (compound A diacetate).[2] These facts indicated that compound C should be compound A monoacetate. Since compound C showed a negative ferric chloride reaction and the carboxyl group was proved to be non-bonded by infrared and ultraviolet spectra, the structure of compound C must be represented as (XV : $R^1=R^4=CH_3$; $R^2=R^3=H$; $H^5=CH_3CO$).

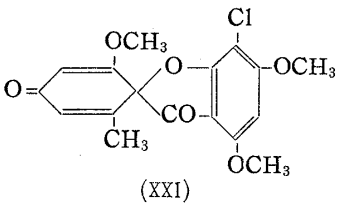
Another component of the mold, compound E, m.p. 147°, comes as pale yellow needles and has a molecular formula, $C_{17}H_{14}O_7$. Its ultraviolet spectrum, λ_{max}^{EtOH} 285 mμ (log ε 4.39), is superimposable with that of geodin[11] (XVIII : X=Cl; R=CH_3). Its infrared spectrum in chloroform solution exhibits absorptions at 3520, 1730, 1712, 1662, and 1638 cm^{-1}, which are assigned respectively to hydroxyl, carbonyl in coumaran-3-one, methoxycarbonyl, carbonyl in 1,4-dienone, and diene. Compound E is optically active, $[\alpha]_D^{15}$ −66° (in EtOH). These observations suggest that compound E might be bisdechlorogeodin (XVIII: X=H; R=CH_3).

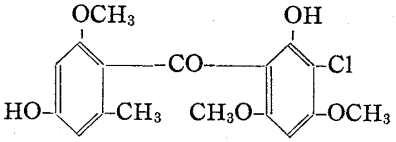
On hydrolysis with sulfuric acid, compound E gave compound A and hydrogenolysis of compound E furnished sulochrin (I). Treatment of compound E with methanol afforded compound D by methanolysis. These reactions were quite similar to those of geodin[7~11] and compound E would be expressed by the formula (XVIII : X=H; R=CH_3). Latest report of Hassall and collaborators[24] showed that they obtained racemic bisdechlorogeodin (4-hydroxy-2′-methoxy-6′-methoxycarbonyl-6-methylgris-2′,5′-diene-3,4′-dione) by oxidation of sulochrin with potassium ferricyanide. The synthetic racemic compound melted at 196°, which is 50° higher than the melting point of levorotatory compound E. Although an attempt to racemise this compound in hydrogen chloride in dry dioxane[11] failed and further trial was impossible due to the scarcity of the sample, both specimens showed quite identical infrared spectra in chloroform solution; thus it would be quite possible to conclude that they are a recemate and an optically active isomer of the same compound (XVIII : X=H; R=CH_3). It should be noted that compound E is levorotatory, while geodin is dextrorotatory.

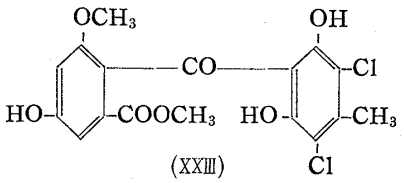
The mold, *Oospora sulphurea-ochracea* v. Beyma, has now been proved to produce one benzophenone (sulochrin) (I), one spiran (compound E) (XVIII), and four diphenyl ether derivatives (compounds A, D, F, and C) (XV). It has been known that *Aspergillus terreus* Thom produces geodin[7~11] (XVIII : R=CH_3; X=Cl), erdin[7~11] (XVIII : R=H; X=Cl), geodoxin[13] (XX), and asterric acid[24] (XV : $R^1=R^4=CH_3$; $R^2=R^3=R^5=H$), and *Penicillium urticae* Bain. (*P. griseofulvum* Dierckx, *P. patulum* Bain.) produces dehydrogriseofulvin[15,17] (XXI) and the corresponding benzophenone derivative[15,17] (XXII), along with griseofulvin[14] and related compounds.[14,15] In *P. estinogenum* Komatsu et Abe, transformation of geodin to dihydrogeodin (XXIII) and *vice versa* has been examined.[12] Biosynthesis of griseofulvin has been studied by Birch[16] to prove the head-to-tail condensation of seven acetate units. Biosynthetic sequence from benzophenone derivatives (I, XXII, XXIII) to diphenyl ethers (XV) through spirocoumaran-3-one (XVIII, XXI), proposed by Barton,[19] Scott,[15,17,18] and Hassall[13,20,24,25] has now been provided with another evidence and suggests more general application.

25) R.F. Curtis, C.H. Hassall, S. Natori, H. Nishikawa : Chem. & Ind. (London), **1961**, 1360.


(XX)


(XXI)


(XXII)


(XXIII)

Experimental*3

C-Methyl Determination of Osoic Acid——Osoic acid, as crystallised from MeOH-water, m.p. 220~222° (decomp.) *Anal.* Calcd. for $C_{15}H_{12}O_8$: C-CH_3, 4.69. Found: C-CH_3, 4.31, 4.52.

Chlorination of Dimethylosoic Acid-I (Compound A)——i) Compound A (350 mg.) in $CHCl_3$ (20 cc.) was treated with Cl_2 in CCl_4 (1*M*, 2.6 cc., 2.6 equiv.) for 3 hr. at a room temperature. The reaction mixture, after washing with water and evaporation, afforded a product, which was recrystallised from MeOH-AcOEt as colorless needles, m.p. 208~209°. Analytical values corresponded to a trichloro compound. *Anal.* Calcd. for $C_{17}H_{13}O_8Cl_3$: C, 45.32; H, 2.90; Cl, 23.55. Found: C, 45.24, 45.43; H, 2.69, 2.79; Cl, 23.63, 23.46.

ii) Many attempts to isolate other chlorination products were unsuccessful by the treatment of compound A with 1 to 8 equivalent of Cl_2 in either $CHCl_3$, CCl_4, or AcOH solution and by separation with silica gel chromatography.

iii) Compound A (350 mg.) in $CHCl_3$ (30 cc.) was treated with SO_2Cl_2 (270 mg., 2 equiv.) in $CHCl_3$ (5 cc.) for 20 hr. After washing and drying, the solvent was evaporated and the residue was recrystallised from MeOH-water to colorless needles, m.p. 221~223° (decomp.). Analytical results showed that the product was a dichloro derivative of compound A but a marked depression of m.p. was observed when fused with geodin hydrate,[10,13] suggesting the position of one of the chlorine atoms is different. *Anal.* Calcd. for $C_{17}H_{14}O_8Cl_2$: C, 48.93; H, 3.38; Cl, 17.00. Found: C, 49.13; H, 3.40; Cl, 17.07, 17.12.

iv) The same reaction with 3 moles of SO_2Cl_2 or chlorination of the dichloro compound of m.p. 221~223° with 1 mole of SO_2Cl_2 afforded the trichloro compound of m.p. 208~209° in a good yield.

v) Further chlorination of the trichloro compound was unsuccessful.

Chlorination of Geodin Hydrate——Geodin hydrate,[10,13] m.p. 203~206°, was prepared from (+)-geodin, isolated from *Penicillium estinogenum* Komatsu et Abe.[12]

i) Chlorination of geodin hydrate with SO_2Cl_2 did not give satisfactory results when $CHCl_3$ was used as the sole solvent.

ii) Geodin hydrate (105 mg.) in $CHCl_3$ (10 cc.) was treated with SO_2Cl_2 (100 mg.) in $CHCl_3$ (0.9 cc.) in the presence of EtOH (0.1 cc.) for 20 hr. Recrystallisation from MeOH-AcOEt afforded the product as colorless needles, m.p. 207~209°. The identity with the trichloro derivative of compound A was established by infrared spectra in Nujol and a mixed fusion. IR (Nujol) cm^{-1}: 3208 (OH), 1724 ($COOCH_3$), 1684 (bonded COOH), 1620, 1575 (phenyl), 1294, 1244, 1147, 1050, 982, 787 (C-Cl).

Chlorination of Pentamethylosoic Acid——i) Pentamethylosoic acid with 2 moles of SO_2Cl_2 in $CHCl_3$ gave, after 24 hr., a dichloro compound, which had m.p. 187~189° after recrystallisation from EtOH. *Anal.* Calcd. for $C_{20}H_{20}O_8Cl_2$: C, 52.30; H, 4.39; Cl, 15.44. Found: C, 52.53; H, 4.78; Cl, 15.87, 15.92.

ii) Further chlorination was unsuccessful in $CHCl_3$. An addition of small amount of EtOH promoted the reaction and a trichloro derivative was obtained as colorless needles, after recrystallisation from MeOH, melted at 146~150°, solidified, and melted again at 164~166°. *Anal.* Calcd. for $C_{20}H_{19}O_8Cl_3$: C, 48.65; H, 3.88; Cl, 21.54. Found: C, 48.83; H, 4.13; Cl, 21.68, 21.82.

Chlorination of Geodin Hydrate Dimethyl Ether Methyl Ester——Chlorination of geodin hydrate dimethyl ether methyl ester,[10,13] m.p. 120°, prepared from geodin hydrate, with SO_2Cl_2 in $CHCl_3$ resulted in the formation of a monochlorinated derivative, which, after crystallisation from

*3 All m.p.s were determined in a sulfuric acid bath and are uncorrected. Infrared spectra were measured as Nujol mull or in $CHCl_3$ solution using a Koken Model 301 Infrared Spectrophotometer. Ultraviolet spectra were determined in EtOH solution in a Cary Model 11 Recording Spectrophotometer.

MeOH, showed identity in melting point, mixed melting point, infrared spectrum with trichloropentamethylosoic acid. IR(Nujol) cm^{-1} : 1735($COOCH_3$), 1602(phenyl), 1276, 1233, 1082, 1059, 867,788(C-Cl).

Norgeodin B from Monomethylanhydro-osoic Acid——Monomethylanhydro-osoic acid[6] (300 mg.) was heated with HI(d=1.7, 20 cc.) and AcOH(10 cc.) for 1 hr.; the reaction mixture was cooled and diluted with water. The crude precipitate, which was sparingly soluble in organic solvents, was recrystallised from ethylene glycol containing a trace of water as colorless needles, m.p. 293~298° (decomp.). The compound gave an orange $FeCl_3$ reaction and a positive Gibbs reaction. *Anal.* Calcd. for $C_{14}H_{10}O_5 \cdot H_2O$: C, 60.87; H, 4.38. Found : C, 60.74; H, 4.46. UV λ_{max}^{EtOH} mμ(log ε) : 227 (4.03), 252(4.20), inf. 282(3.89), 309(3.85), 360(3.43). IR(Nujol) cm^{-1} : 3532, 3217(OH), 1625(xanthone CO).

The product (400 mg.) was treated with Me_2SO_4 (4 cc.) and 2*N* NaOH (32 cc.) in Me_2CO (8 cc.) for 1 hr. The precipitate was collected and recrystallised from EtOH to colorless needles, m.p. 170~183°. Further purification through a column of alumina in benzene solution gave colorless needles of m.p. 198~200°. *Anal.* Calcd. for $C_{17}H_{16}O_5$: C, 67.99; H, 5.32; $3CH_3O$, 31.00. Found : C, 67.59; H, 5.49; CH_3O, 32.18.

The properties of these compounds showed agreement with norgeodin-B and its trimethyl ether,[10] which were now proved to be 1-methyl-3,5,7-trihydroxyxanthone and its trimethyl ether.

Chlorination of Sulochrin——Sulochrin (500 mg.) in $CHCl_3$ (30 cc.) was chlorinated with molecular Cl_2 in CCl_4 (1*M*, 4.0 cc., 2.6 equiv.). The crude reaction product was passed through a column of silica gel in benzene solution; the main yellow eluate was collected and the evaporated residue was recrystallised from MeOH-water as yellow needles of m.p. 217~219°. *Anal.* Calcd. for $C_{17}H_{13}O_7Cl_3$: C, 46.87; H, 3.01; Cl, 24.42. Found : C, 46.83, 46.78; H, 2.97, 3.23; Cl, 24.68, 24.89.

Respective Identification of Dimethylosoic Acid-I (Compound A), Trimethylosoic Acid-I (Compound D), and Monomethylosoic Acid (Compound F) with Asterric Acid, Methyl Asterrate, and Demethylasterric Acid——Compound A was recrystallised from MeOH-water as colorless needles, m.p. 211~212°. A mixed fusion with asterric acid,[24] m.p. 208~214°, melted at 208~214°.

Compound D was recrystallised from MeOH as colorless needles, m.p. 188~189°. The identity with methyl asterrate,[24] m.p. 184~186°, was confirmed by infrared spectra in Nujol and a mixed fusion.

Compound F was recrystallised from water to colorless needles of m.p. 237~241°(decomp.). A mixed fusion and infrared spectra in Nujol showed the identity with demethylasterric acid,[24] m.p. 239~242°(decomp.).

Compound C——Crystallisation from MeOH gave colorless needles of m.p. 198~199°(with effervescence). It gave a negative $FeCl_3$ reaction and is soluble in $NaHCO_3$ solution. Analytical values reported in the previous paper[1] also agree with the following molecular formula. *Anal.* Calcd. for $C_{19}H_{18}O_9$: C, 58.46; H, 4.65; $2CH_3O$, 15.90; 1COOH, 11.54; $1CH_3CO$, 11.04. Found : C, 58.83, 58.68; H, 4.94, 4.95; CH_3O, 15.97, 15.88; COOH, 11.90; CH_3CO, 12.98. IR (Nujol) cm^{-1} : 3333 (OH), 1761 (CH_3CO), 1723 ($COOCH_3$), 1692(non-bonded COOH). UV : λ_{max}^{EtOH} mμ(log ε) : 213(4.80), 313(3.81).

Formation of Compound F from Compound C——Compound C(300 mg.) in methanolic KOH(10%, 20 cc.) was refluxed for 2 hr. The precipitate obtained by acidification after cooling was recrystallised from water to colorless needles of m.p. 239~242°(decomp.), which showed no depression of m.p. when fused with compound F.

Acetylation of Compound C——Compound C(10 mg.) was treated with Ac_2O (3 cc.) and pyridine (3 cc.) for 24 hr. The product was crystallised from MeOH-water as colorless prisms, m.p. 143~146°, which was proved to be identical with compound A diacetate[2] by a mixed fusion.

Compound D Diacetate——With Ac_2O-H_2SO_4, compound D gave diacetate, which was recrystallised from MeOH as colorless prisms, m.p. 125~126°. *Anal.* Calcd. for $C_{22}H_{22}O_{10}$: C, 59.19; H, 4.97. Found : C, 59.04; H, 4.73.

Compound E——The pale yellow needles, after crystallisation from EtOAc-light petroleum (b.p. 60~80°), had m.p. 147~148°. It is insoluble in $NaHCO_3$ solution and soluble in NaOH solution. It gave a negative $FeCl_3$ reaction. A molecular formula, $C_{17}H_{14}O_7$, was reported in the previous paper.[2] $[\alpha]_D^{15}$ −66°(c=0.53, EtOH). IR(Nujol) cm^{-1} : 3450(OH), 1729(CO in spiro-coumaran), 1710 (COOMe), 1660(1,4-dienone), 1626(broad) (ene and phenyl). IR($CHCl_3$) cm^{-1} : 3520, 1730, 1712, 1662, 1638, 1612. UV : λ_{max}^{EtOH} 285 mμ (log ε 4.39).

The mixed fusion of compound E, m.p. 145~147°, $[\alpha]_D$ −66°, with 4-hydroxy-2′-methoxy-6′-methoxycarbonyl-6-methylgris-2′,5′-diene-3,4′-dione,[24] m.p. 188~191°, $[\alpha]_D$ 0°, softend around 142° and melted at 184~191°. The infrared spectra of the optically active and the racemic spiro compounds were identical in all regions in $CHCl_3$.

Hydrolysis of Compound E with Sulfuric Acid——Compound E was dissolved in H_2SO_4, which was added to water to form a precipitate. It was treated with Et_2O and shaken with $NaHCO_3$ solution. After acidification and extraction with Et_2O, the extract was evaporated to leave a colorless solid, which was crystallised from MeOH to colorless needles of m.p. 210~212°; the identity of the product with compound A was established by a mixed fusion and infrared spectra in Nujol.

An attempt to racemise compound E with 2% HCl in dry dioxane[11] failed and compound A was isolated from the reaction mixture.

Methanolysis of Compound E——Compound E (10 mg.) was dissolved in MeOH (10 cc.) and kept standing at a room temperature for 10 days. Evaporation of MeOH and addition of water gave colorless needles, m.p. 187~189°, which showed the identity with compound D by a mixed fusion and infrared spectra in Nujol.

Hydrogenolysis of Compound E ——Compound E (90 mg.) in EtOH (23 cc.) was hydrogenated in the presence of Pd-C (10%, 80 mg.). An absorption of 1 mole of H_2 occurred immediately. Evaporation of EtOH solution in a reduced pressure and an addition of water afforded colorless needles of m.p. 140~210°, which was purified by chromatography in benzene on acid-washed alumina. From benzene-EtOH (99:1) eluate, an amorphous pale yellow solid was obtained, which was recrystallised from MeOH-water as pale yellow needles of m.p. 251~255° (decomp.). Mixed fusion with a sample of sulochrin melted at 250~254° (decomp.) and the infrared and ultraviolet spectra showed identity with those of sulochrin. IR (Nujol) cm^{-1} : 3512, 3358 (OH), 1698 (COOMe), 1639 (conj. C=O), 1613, 1593, 1250, 1202, 1068, 1002, 917, 817. UV λ_{max}^{EtOH} mμ (log ε) : inf. 220 (4.67), 283 (4.29), inf. 325 (3.92).

Methanolysis of (+)-Geodin——(+)-Geodin[12] was dissolved in MeOH and kept standing at a room temperature for 5 days. Evaporation and addition of water afforded crystalline deposit which was recrystallised from MeOH-water as colorlees needles of m.p. 153~154°.[12] It is insoluble in $NaHCO_3$ and soluble in NaOH. UV λ_{max}^{EtOH} mμ (log ε) : 208 (5.09), inf. 300 (4.05), 321 (4.13). *Anal.* Calcd. for $C_{18}H_{16}O_8Cl_2$: C, 50.13; H, 3.74; Cl, 16.44; $3CH_3O$, 21.59. Found : C, 49.80; H, 3.45; Cl, 16.85; CH_3O, 21.28.

Geodin hydrate was treated with excess of diazomethane for a few min. and the mixture was treated with a drop of AcOH. The residue, after evaporation, was recrystallised from MeOH-water as colorless needles of m.p. 149~153°, which was identical in melting point and mixed melting point with the above methanolysis product.

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Summary

The structures of the metabolites of *Oospora sulphurea-ochracea* v. Beyma, compounds A, D, and F (dimethylosoic acid-I, trimethylosoic acid I and monomethylosoic acid), were proved to be diphenyl ethers (XV : $R^1=R^4=CH_3$; $R^2=R^3=R^5=H$; $R^1=R^2=R^4=CH_3$; $R^3=R^5=H$; and $R^4=CH_3$; $R^1=R^2=R^3=R^5=H$) and compound A was proved to be identical with asterric acid[24] produced by *Aspergillus terreus* Thom.

Other metabolite of the mold, compounds C and E, were respectively expressed by compound A monoacetate (XV : $R^1=R^4=CH_3$; $R^2=R^3=H$; $R^5=COCH_3$) and bisdechlorogeodin (XVIII : X=H; R=CH_3).

Biogenetic sequence of these metabolites was discussed.

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