the solution gave a similar absorption as that of II in concentrated sulfuric acid with potassium nitrate (Fig. 3). Therefore, in this case, the ESR spectrum of the solution indicated the formation of dibenzo-*p*-dioxin-2,7-disulfonic acid (II) by standing dibenzo-*p*-dioxin (I) in concentrated sulfuric acid at room temperature.

Experimental

Electron Spin Resonance Spectra——A JES-3B Spectrometer (Japan Electron Optics Laboratory Co., Ltd.) was used with 100 Kc. field modulation. In all spectra, the field sweep increased in the same rate from left to right on the figures and a modulation amplitude of 0.1 gauss. An aqueous solution of potassium peroxylamine disulfonate[4] was used as a standard of the magnetic field and the g-value.

The author express his gratitude to Prof. M. Tomita, Faculty of Pharmaceutical Sciences, Kyoto University, for his kind encouragement and advices. He is also appreciative of the encouragement of Prof. H. Takaki, Dr. Y. Deguchi, and Mr. Y. Nakai, Faculty of Science, Kyoto University.

Summary

Dibenzo-*p*-dioxin-2,7-dicarboxylic acid (III) in concentrated sulfuric acid with potassium nitrate gave an electron spin resonance spectrum which was considerably similar to that of dibenzo-*p*-dioxin-2,7-disulfonic acid (II).

Therefore, the spectra indicated that the distributions of the spin densities of the odd electron were quite similar in both cases.

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30. Tadasu Tanaka : Synthesis of the Compounds Containing Grisan Ring.*1

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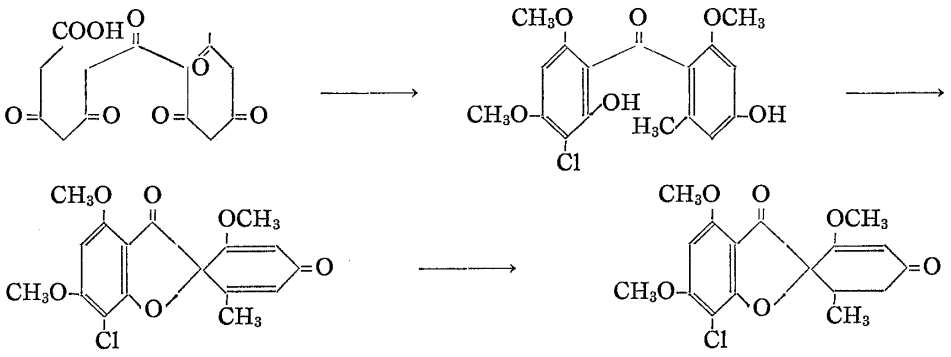
In 1958, Birch and his co-workers[1] demonstrated that the mould metabolite griseofulvin was derived from β-poly carbonyl system through "head to tail" condensation of seven units of acetic acid.



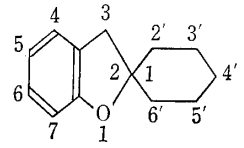
*1 The paper peported at the 82nd annual meeting of the Pharmaceutical Society of Japan, Nov. 3rd. 1962.

*2 960, Kashimacho, Higashiyodogawa-ku, Osaka (田中 雅).

1) A. J. Birch, R. A. Massy Westropp, R. W. Richards, H. Smith : J. Chem. Soc., **1958**, 360.

Later, English workers[2] proposed the more detailed biogenesis of griseofulvin based on the fact that the new benzophenone and spirodienone were isolated togather with griseofulvin from *Penicillium patulum* culture.

Total syntheses of griseofulvin, simulating its biogenesis have already been achieved.[3,4] As a preliminary to an independent synthesis of griseofulvin, the present author has undertaken a synthesis of the grisan[5] skeleton from a simple β-poly carbonyl compound such as 4-(*o*-hydroxybenzoyl)-cyclohexan-1,3-idone (Ⅳ).



grisan

In 1958, Hauser and Harris reported[6] a general synthetic method of straight chained 1,3,5-triketones by the Claisen-type condensation of dipotassio salt of β-diketones such as acetylacetone and benzoylacetone with methyl aroylates in liquid ammonia.

This procedure was applied to the cyclic β-diketones for the preparation of Ⅳ. Thus cyclohexan-1,3-dione (Ⅰ : R_1=H) was treated with two molar equivalents of sodium amide in liquid ammonia. The resultant disodio salt was condensed with one-half molar equvalent of methyl *o*-methoxymethoxy benzoate (Ⅱ : R_2=CH_2OCH_3) to give 4-(*o*-methoxymethoxy benzoyl)-cyclohexan-1,3-dione(Ⅲ : R_1=H, R_2=CH_2OCH_3). This compound showed a deep green color with ferric chloride in methanol and had infrared bands at 2500 cm^{-1} (a very broad band due to conjugate chelation),[7] 1580 (enol band)[7] and 1670 cm^{-1} (aromatic C=O), characteristic of 4-aroylcyclohexan-1,3-diones.[8]

Hydrolysis of Ⅲ (R_1=H, R_2=CH_2OCH_3) with dilute mineral acid at room temperature[9] gave Ⅳ, but only with a low overall yield.

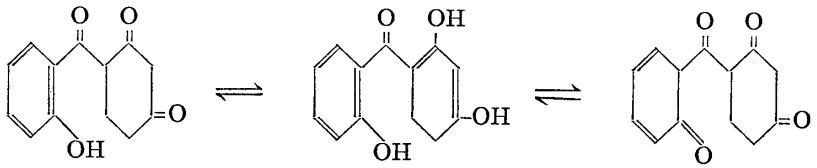
Interaction of sodium salt of methyl salicylate (Ⅱ : R_2=Na) and (Ⅰ : R_1=H) under similar conditions, which gave Ⅳ in 50~60% yield in one step, was found to be a better alternative for the same purpose.

When 5-methyl derivate (Ⅰ : R_1=CH_3) was reacted with Ⅱ (R_2=CH_2OCH_3), Ⅲ (R_1=CH_3, R_2=CH_2OCH_3) was easily obtained. But in contrast to Ⅳ no β-tetraketone*3 was isolated when Ⅲ (R_1=CH_3, R_2=CH_2OCH_3) was hydrolyzed with mineral acid. The only product obtained was 1-methyl-1,2,3,4-tetrahydro-3,9-dioxoxanthene.

In order to prove the structure of Ⅳ, this was dehydrogenated by treating with N-bromosuccinimide in carbon tetrachloride to give 3-hydroxy-9-xanthenone (Ⅵ).

The infrared spectrum and melting point of Ⅵ were the same with those of the one prepared by the Ullmann's method.[10] Thus it was proved that *o*-hydroxybenzoyl group was introduced at the 4-position of cyclohexan-1,3-dione, but not at the 2-position. The

*3 For instance, the following β-tetracarbonyl or diosphenol system may be assumed for the tautomeric keto-enol form of Ⅳa.



2) W. J. MacMaster, A. I. Scott, S. Trippett : J. Chem. Soc., **1960**, 4628. A. Rhodes, B. Boothroyd, M. P. Mcgonagle, G. A. Somerfield : Biochem. J., **81**, 28 (1961).
3) A. C. Day, J. Nabney, A. I. Scott : J. Chem. Soc., **1961**, 4067.
4) D. Taub, C. H. Kuo, H. L. Slates, N. L. Wendler : Tetrahedron., **19**, 1 (1963).
5) J. F. Grove, J. MacMillan, T. P. C. Mullholland, M. A. T. Rogers : J. Chem. Soc., **1952**, 3979.
6) C. H. Hauser, T. H. Harris : J. Am. Chem. Soc., **80**, 6360 (1958).
7) R. S. Rusmussen, D. D. Tunicliff, R. R. Brittain : J. Am. Chem. Soc., **71**, 1068 (1949). K. Kotera : Yakugaku Zasshi, **81**, 442 (1961).
8) Reported at the Kinki Branch meeting of the Pharmaceutical Society of Japan, Nov., 23, 1962.
9) F. B. Laforge : J. Am. Chem. Soc., **55**, 3045 (1933).
10) A. Bayer : Ann., **372**, 99 (1910); F. Ullmann, W. Denzler : Ber., **39**, 4339 (1906).

ultraviolet spectrum of Ⅳ showed a simple summation of the absorptions of *o*-hydroxyacetophenone and cyclohexan-1,3-dione as shown in Fig. 2. UV λ_{max}^{MeOH} mμ (log ε) : 245 (4.06), 280 (3.96), 316 (3.62).

Its infrared spectrum, however, manifested rather unexpectedly a sharp -OH absorption at 3280 cm^{-1}, as shown in Fig. 1, different in type from the band attributable to the intramolecular chelation of *o*-hydroxyphenyl ketone type (3200~2500 cm^{-1} very broad).[11] The carbonyl absorptions had its bands at 1717 and 1690 cm^{-1}. The former was ascribable to the normal aliphatic carbonyl, and the later to the normal aryl ketone

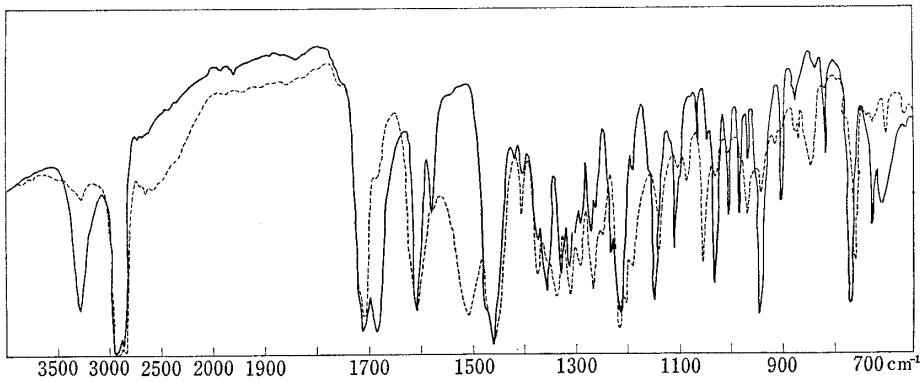


Fig. 1. Infrared Spectra in Nujol

——— 4-(*o*-hydroxy)benzoyl cyclohexan-1,3-dione (Ⅳ)
----- grisan-3,2′,4′-trione (Ⅷ)

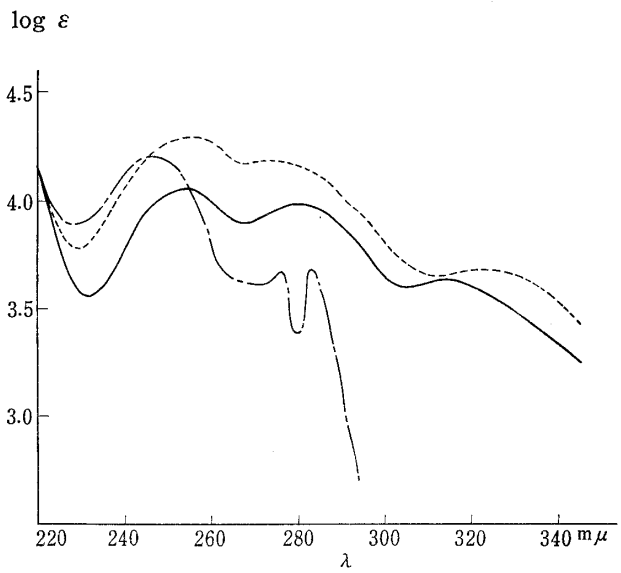


Fig. 2. Ultraviolet Spectra in Methanol

——— 4-(*o*-hydroxy)benzoyl cyclohexan-1,3-dione (Ⅳ)
----- grisan-3,2′,4′-trione (Ⅷ)
-·—·- 5-benzofuranyl 2-pentenoic acid (XVIII)

type (1700~1680 cm^{-1}) rather than *o*-hydroxyphenyl ketone type (1655~1635 cm^{-1}).[12]

These inconsistent spectroscopic data seem to suggest the presence of a ring-chain isomerism (Ⅳa) ⇌ (Ⅳb) in Ⅳ.

Ⅳ was easily dehydrated by heating with dilute mineral acid to yield 1,2,3,4-tetrahydro-3,9-dioxoxanthene (Ⅴ), and gave its oxime (Ⅶ) when treated with hydroxylamine in pyridine. These results could be well explained by adapting the ring form (Ⅳb) for (Ⅳ). Nevertheless, solubility in aqueous sodium carbonate, the characteristic green ferric reaction, and the analysis of ultraviolet spectrum led the author to the conclusion that the chain form (Ⅳa) must also be considered to exist.

Efforts were now made to prepare spirocoumaranone from the foregoing β-tetraketone (Ⅳa) by intramolecular phenyl ether linkage.

11) L. J. Bellamy : "The Infrared Spectra of Complex Molecules" 2nd Ed., 96 (1958), Methuen & Co., Ltd., London.
12) *Idem* : *Ibid.*, 132 (1958). Methuen Co., Ltd. London.

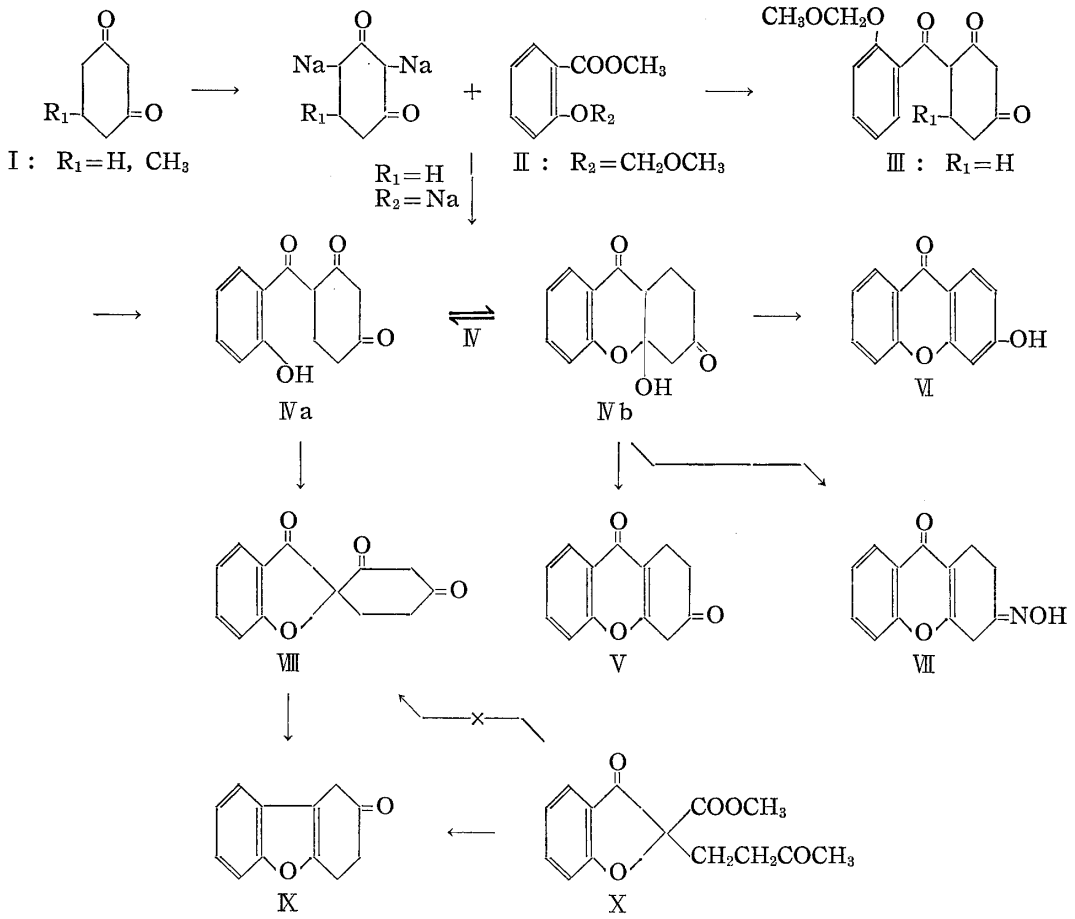
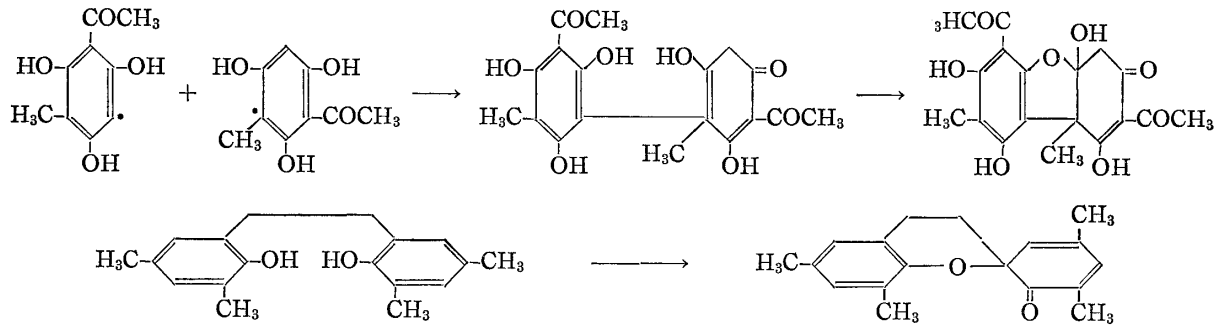


Chart 1.

A couple of such examples by the oxidative ring closure of phenols were cited. Barton and his collaborators[13] succeeded to obtain usnic acid by the intermolecular oxidative condensation of methylphloroacetophenone with potassium ferricyanide in aqueous sodium carbonate solution.

Intramolecular condensation of *o,o'*-dioxydimesityl with the same reagent to furnish the spirodienone was also reported.[14]



By applying the Barton's ferricyanide oxidation procedure to the supposed diosphenol structure of IVa, grisan-3,2',4'-trione (VIII) was readily obtained, which, in conformity with its structure, had an acidic character (soluble in sodium bicarbonate solution) and gave orange yellow ferric reaction. Its ultraviolet spectrum showed a summation type

13) D. H. R. Barton, A. M. Deflorin, E. E. Edwards : J. Chem. Soc., **1956**, 530.
14) K. Fries, E. Brandes : Ann., **542**, 55 (1939).

of the absorption of coumaran-3-one and cyclohexan-1,3-dione, UV λ_{max}^{MeOH} mμ (log ε): 254 (4.30), 275 (4.19), 326 (3.69) and the infrared spectrum had bands at 2500 cm^{-1} (very broad band, conjugate chelation), at 1615, 1515 cm^{-1} (enol bands), and at 1720 cm^{-1} (coumaran-3-one).

When refluxed with aqueous sodium hydroxide, a neutral substance was obtained. This compound, presumed to be 1,2,3,4-tetrahydro-2-oxodibenzofuran (Ⅸ), was formed through cleavage of the C-ring of Ⅷ by the agency of alkali, followed by subsequent cyclization. Its unique type of ultraviolet spectrum for benzofuran[15] and infrared band at 1715 cm^{-1} (aliphatic C=O) were also consistent as Ⅸ.

Moreover this was identified with the compound prepared by the Henecka's[16] method. The analogous result was reported by Grove and his co-workes[5] in their studies of the structure of griseofulvin.

Cyclization to the griseofulvic acid and its dechloro derivative from the corresponding 2-methoxycarbonyl-2-(pentan-4-on-2-yl)-coumaran-3-ones using sodium methoxyde were achieved by Swiss workers.[17,18] But attempts to prepare Ⅷ from 2-methoxycarbonyl-2-(butan-3-onyl)coumaran-3-one (Ⅹ) by the analogous method failed.

Methylation of Ⅷ with diazomethane afforded two isomeric methyl ethers, the one of m.p. 143° (Ⅺb) and the other of m.p. 123° (Ⅺa) in a ratio of about 6:4. In order to decide the structure of both isomers, they were derived to the simpler grisandiones. Reduction of Ⅺa with sodium borohydride in methanol followed by acidification to pH 4~4.5 gave 3-hydroxygris-2′-en-4′-one (Ⅻa). Catalytic reduction of Ⅻa in the presence of 10% palladium-charcoal afforded 3-hydroxygrisan-4′-one (XIIIa), which on being oxidized with chromic acid in acetic acid gave rise to grisan-3,4′-dione (XIVa) already synthesized by McClosky.[19] The author also prepared the same compound by other method. Michael condensation of coumaran-3-one (XV) with two moles of methyl acrylate using Triton B catalyst yielded 2:2-di-(2-methoxycarbonylethyl)-coumaran-3-one (XVI), which was hydrolysed to the corresponding dibasic acid (XVII).

This was refluxed with acetic anhydride containing catalytic amount of potassium cyanide[20] to give XIVa, which was identical with the compound mentioned above.

On the other hand, the substance (Ⅺb) of m.p. 123° was treated as above Ⅺa→XIVa to give grisan-3,2′-dione (XIVb). When the intermediate (Ⅻb) was warmed with mineral acid, an acidic substance $C_{13}H_{12}O_3$ was obtained.

It had the benzofuran type[15] ultraviolet spectrum, λ_{max}^{MeOH} mμ (log ε); 248 (4.22), 276 (3.69). 283 (3.71) and had infrared bands at 2600, 1695 cm^{-1} due to the $\alpha\beta$-unsaturated carboxylic acid. Hence 5-benzofuranyl-2-pentenoic acid (XVIII) was proposed as its structure which was presumably produced from Ⅻb under the fission of C-ring followed by loss of water. XVIII was readily reduced catalytically to yield the saturated acid (XIX). Synthetic support for XIX was provided as follows.

γ-Carbethoxybutyroyl chloride (XX) was converted into ethyl 6-bromo-5-oxocaproate[21] (XXII) *via* the diazoketone (XXI) by the usual method.

When XXII was refluxed with sodium salt of salicylic aldehyde in benzene solution, condensation and subsequent cyclization took place at once to furnish XXIII, which was reduced according to Wolf-Kishner method to yield XIX, ester group being hydrolyzed simultaneously.

15) R. Gaerter : J. Am. Chem. Soc., **73**, 4401 (1951).
16) H. Henecka : Chem. Ber., **81**, 206 (1948).
17) A. Brossi, M. Baumann, M. Gereke, E. Kyburz : Helv. Chim. Acta., **43**, 2071 (1960).
18) M. Gereke, E. Kyburz, C. V. Planta, A. Brossi : *Ibid.*, **45**, 2241 (1962).
19) P. McCloskey : J. Chem. Soc., **1958**, 4733.
20) F. C. Uhle : J. Am. Chem. Soc.. **71**, 761 (1949).
21) A. Businger : "Jubilee. Vol. Emil. Barell," **1946**, 137. (Chem. Abstr., **41**, 3791 (1947)).

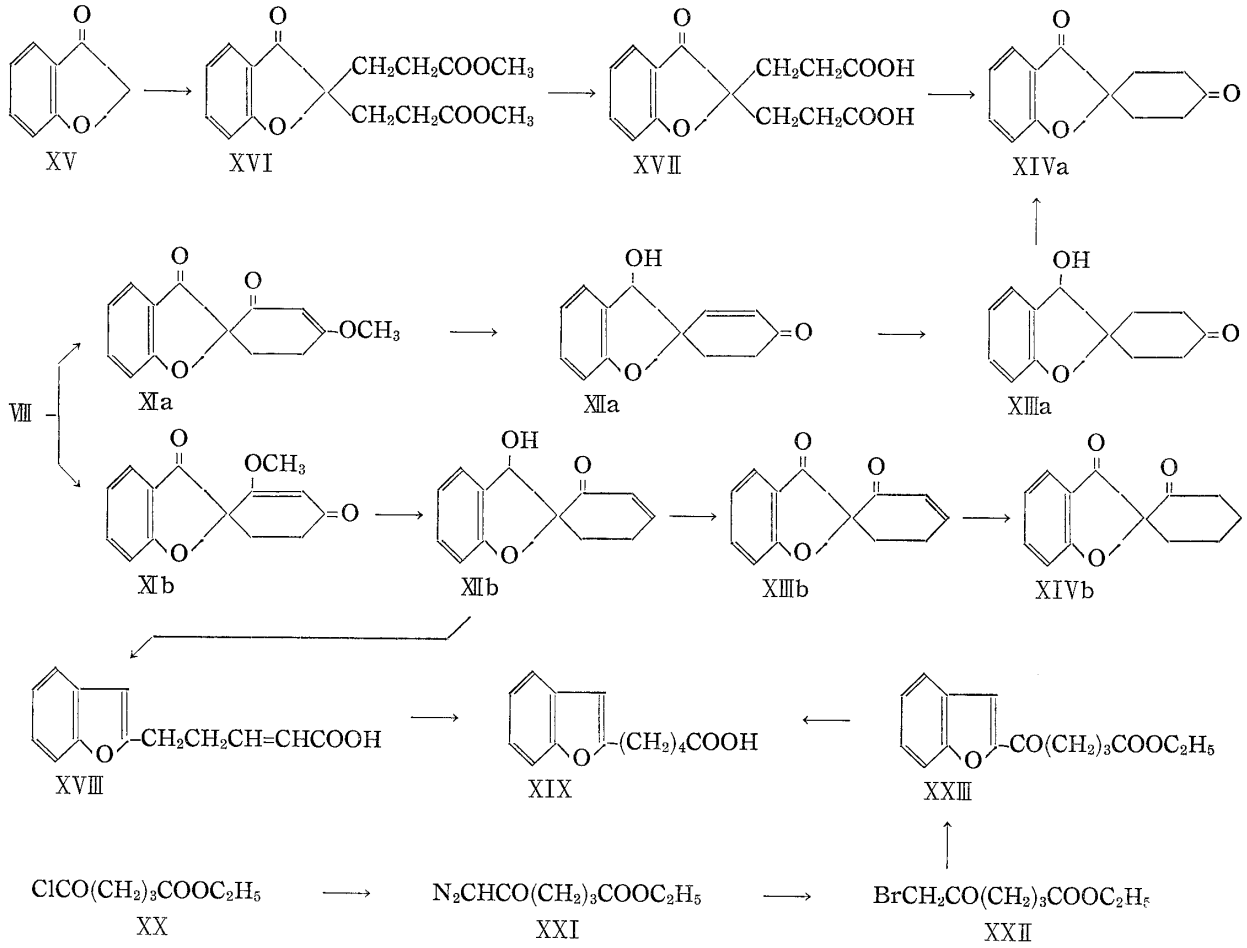


Chart 2.

This compound was identified with the above substance derived from XIIb through mixed melting point test and infrared spectra.

Experimental

***o*-Methoxymethoxybenzoylation of 1,3-Cyclohexanedione and its 5-Methyl Analog**——To a stirred suspension of 0.4 mole of $NaNH_2$ in 400 ml. of liquid NH_3 was added in small portions 0.2 mole of the β-diketone. After stirring for 45 min. the resulting faint green suspension was considered to contain 0.2 mole of the disodio salt.

To the stirred suspension was added a solution of 0.1 mole of methyl *o*-methoxymethoxybenzoate in 20 ml. of anhyd. Et_2O as fast as possible. After stirring for 1 hr., liquid NH_3 evaporated as an equal volume of Et_2O was added. The etherial suspension was poured into excess of cold, dil. AcOH. The etherial layer was separated and the aqueous layer was extracted with AcOEt and the whole organic layer was dried over anhyd. Na_2SO_4. The solvent was removed *in vacuo*, and the dark gummy residue was crystallized from AcOEt-petr. ether to give the 4-(*o*-methoxymethoxybenzoyl)-5-alkyl-1,3-cyclohexanedione.

4-(*o*-Methoxymethoxybenzoyl)-1,3-cyclohexanedione (III : R_1 =H, R_2=$CH_2O\cdot CH_3$)——Colorless fine needles, m.p. 116~118°. Yield : 10.2 g. (36.9%). *Anal.* Calcd. for $C_{15}H_{16}O_5$: C, 65.21; H, 5.84. Found : C, 65.38; H, 5.70. UV λ_{max}^{MeOH} mμ (log ε) : 258 (4.08), 281 (4.26).

4-(*o*-Methoxymethoxybenzoyl)-5-methyl-1,3-cyclohexanedione (III : R_1=CH_3, R_2=CH_2OCH_3)——Colorless fine needles, m.p. 114~116°. Yield : 11.8 g. (41.0%). *Anal.* Calcd. for $C_{16}H_{18}O_5$: C, 66.19; H, 6.25. Found : C, 66.22; H, 6.25. UV λ_{max}^{MeOH} mμ (log ε) : 254 (4.23), 279 (4.12).

4-(*o*-Hydroxybenzoyl)-1,3-cyclohexanedine (IV). A) By *o*-Hydroxybenzoylation of I (R_1=H)——Seventeen and four-tenths grams of Na salt of methyl salicylate (II : R_2=Na) was added to a stirred suspension of 0.2 mole of disodio-1,3-cyclohexanedione in 400 ml. or liquid NH_3 according to the foregoing method.

After stirring for 1 hr. the NH_3 was evaporated at the room temperature as a 400 ml. of Et_2O was added. The Et_2O suspension was poured onto crushed ice containing 30 ml. of AcOH. The separated precipitate was filtered, washed with H_2O, and crystallized from dioxane to give 12.0 g. (51.5%) of colorless rhombs, m.p. 185° (decomp.). *Anal.* Calcd. for $C_{13}H_{12}O_4$: C, 67.23; H, 5.27. Found: C, 66.93; H, 5.20.

B) By Hydrolysis of III (R_1=H, R_2=$CH_2O\cdot CH_3$)——A solution of 1.2 g. of III (R_1=H, R_2=CH_2-OCH_3) in 5 ml. of 5% HCl and 5 ml. of EtOH was allowed to stand at 15~20° for 30 min. The separated crystals were collected, washed with H_2O and recrystallized from dioxane to give 0.2 g. of IV. This compound was identical with the one prepared in A) by mixed melting point determination and comparison of infrared spectra.

1-Methyl-1,2,3,4-tetrahydro-3,9-dioxoxanthene——A solution of 2.4 g. of III (R_1=CH_3, R_2=CH_2O-CH_3) in 10 ml. of 10% H_2SO_4 and 15 ml. of MeOH was refluxed for 1 hr., then MeOH was evaporated *in vacuo*. The separated oil solidified after being kept at room temperature. Crystallization from EtOH gave 1.6 g. of pale yellow plates, m.p. 132~134°. *Anal.* Calcd. for $C_{14}H_{12}O_3$: C, 73.64; H, 5.30. Found: C, 73.44; H, 5.30. IR ν_{max}^{Nujol} cm^{-1}: 1730 (aliphatic C=O), 1640 (aromatic C=O).

The monooxime prepared in the usual way was crystallized from EtOH in colorless rhombs, m.p. 176~178°. *Anal.* Calcd. for $C_{14}H_{13}O_3N$: C, 69.12; H, 5.39; N, 5.76. Found: C, 68.86; H, 5.45; N, 5.54.

1,2,3,4-Tetrahydro-3,9-dioxoxanthene (V)——This compound was obtained from III (R_1=H, R_2=CH_2-OCH_3) or IV by the same operation as described above. V was crystallized from AcOEt in yellow plates, m.p. 160~161°. *Anal.* Calcd. for $C_{13}H_{10}O_3$: C, 72.89; H, 4.71. Found: C, 72.59; H, 4.72. IR ν_{max}^{Nujol} cm^{-1}: 1725, 1640. UV λ_{max}^{MeOH} mμ (log ε): 226 (4.33), 243 (3.99), 263 (3.79), 299 (3.91).

The monooxime crystallized from EtOH in colorless needles, m.p. 213~214° (decomp.). This compound was identical with the one prepared in the following procedure by mixed melting point detrmination and comparison of IR spectra.

To a solution of 0.7 g. of IV in 7 ml. of pyridine was added a solution of 0.7 g. of $H_2NOH\cdot HCl$ in 0.5 ml. of H_2O, and the mixture was kept at room temperature for 2 days, then poured into excess of cold, dil. AcOH. The separated precipitate was filtered, washed with H_2O and crystallized from dioxane to give 0.4 g. of the monoxime (VII) in colorless needles, m.p. 213~214° (decomp.). *Anal.* Calcd. for $C_{13}H_{11}O_3N$: C, 68.11; H, 4.84; N, 6.11. Found: C, 68.14; H, 4.87; N, 6.14. IR ν_{max}^{Nujol} cm^{-1}: 3250 (OH), 1635 (aromatic C=O), 1605 (C=N).

3-Hydroxy-9-oxoxanthene (VI)——To a solution of 1.16 g. of IV in 40 ml. of CCl_4 was added 0.93 g. of N-bromosuccinimide and 0.02 g. of benzoylperoxide. The mixture was refluxed for 6 hr. and kept overnight in a refrigerator. The solid was collected and rinsed with cold MeOH thoroughly. Evaporation of MeOH washings gave crude VI, which was recrystallized 3 times from aqueous-MeOH to give 0.4 g. of colorless sandy crystals, m.p. 244~246°. *Anal.* Calcd. for $C_{13}H_8O_3$: C, 73.58; H, 3.80. Found: C, 73.31; H, 4.11.

VI was identical with the compound prepared by the Ullmann's method.

Grisan-3,2',4'-trione (VIII)——Five and eight-tenths grams of IV was dissolved in a solution of 7.9 g. of anhyd. Na_2CO_3 in 300 ml. of H_2O and allowed to stand for about 2 hr.[*4] To the solution, with gentle stirring to prevent from excess foaming, was added dropwise a solution of 16.5 g. of $K_3Fe(CN)_6$ in 400 ml. of H_2O at 15~18°. After stirring for 2 hr. at the same temperature, the dark green solution was acidified to congo red with 20% H_2SO_4. A little amorphous precipitate first separated was filtered off and the filtrate was allowed to stand over night in a refrigerator.

Separated crystals were collected, washed with H_2O and dried in a vacuum desiccator.

It was crystallized from AcOEt-Petr. ether to give 4.0 g. of colorless rhombs, m.p. 172° (decomp.). *Anal.* Calcd. for $C_{13}H_{10}O_4$: C, 67.82; H, 4.38. Found: C, 67.93; H, 4.56.

Hydrolysis of VIII with aq. Sodium Hydroxide——A solution of 650 mg. of VIII in 10 ml. of 2% NaOH was refluxed for 1 hr. Original clear solution became turbide gradually and the crystals separated when the mixture was allowed to stand at room temperature. The mixture was extracted with Et_2O and the etherial layer was washed with 2% NaOH, with H_2O, and dried over andyd. K_2CO_3. The solvent was removed and the solid residue was crystallized from EtOH to give 280 mg. of 2-oxo-1,2,3,4-tetrahydrodibenzofuran (IX) in colorless plates, m.p. 106~107°.

Methylation of VIII——A suspension of 6.0 g. of VIII in 300 ml. of Et_2O was treated at 0~5° with an excess of etherial diazomethane. After standing 24 hr. at 5~10°, excess of diazomethane and Et_2O was removed *in vacuo*. The residual solid was washed several times with cold Et_2O, then recrystallized from EtOH to give 3.0 g. of 4'-methoxygris-3'-en-3,2'-dione (XIb) in colorless needles, m.p. 143~144°. *Anal.* Calcd. for $C_{14}H_{12}O_4$: C, 68.84; H, 4.95. Found: C, 68.62; H, 4.96. IR ν_{max}^{Nujol} cm^{-1}: 1715 (coumaranone C=O), 1655 (αβ-unsaturated C=O), 1610 (C=C). UV λ_{max}^{MeOH} mμ (log ε): 250 (4.45), 326 (3.72).

*4 In all cases out of 6 experiments, quite low yields (0~5%) of VIII were recorded when the oxidation started as soon as IV was dissolved in Na_2CO_3 solution.

The combined Et_2O washings were distilled and the residue was crystallized from AcOEt-petr. benzin 4 times to give 2.1 g. of 2′-methoxygris-2′-en-3,4′-dione (XIa) in colorless fine needles, m.p. 123～124°. *Anal.* Calcd. for $C_{14}H_{12}O_4$: C, 68.84; H, 4.95. Found : C, 68.88; H, 4.99. IR ν_{max}^{Nujol} cm^{-1}: 1710, 1665, 1600. UV λ_{max}^{MeOH} mμ (log ε) : 254 (4.47), 328 (3.72).

Amination of XIa——Fourhandred and eighty milligrams of XIa was dissolved in 5 ml. of 25% NH_3 solution in MeOH and allowed to stand for 3 days.

The solvent was distilled *in vacuo*, and the residue was crystallized from aq. MeOH to give 320 mg. of 4′-aminogris-3′-en-3,2′-dione in yellow plates, m.p. 246～248°. *Anal.* Calcd for $C_{13}H_{11}O_3N$: C, 68.11; H, 4.84; N, 6.11. Found : C, 67.97; H, 4.81; N, 6.16. IR ν_{max}^{Nujol} cm^{-1} : 3400, 3170 (NH_2), 1710 (aromatic C=O), 1660 (αβ-unsaturated C=O), 1615 (C=C).

The corresponding amino derivative was not obtained by the amination of XIb.

3-Hydroxygris-2′-en-4′-one (XIIa)——To a stirred solution of 0.55 g. of XIa in 20 ml. of MeOH was added a solution of 0.3 g. of $NaBH_4$ in 15 ml. of MeOH at 15～20°. After 2 hr., 60 ml. of H_2O was added and the solution was neutralized to pH 4～4.5 with 10% HCl. The solvent was distilled *in vacuo* under 40° untill the white crystals begain to separate. After standing overnight in a refrigerator, the crystals were collected, washed with H_2O, and dried in a vacuum desiccator. Recrystallization from AcOEt-petr. benzin afforded 0.38 g. of XIIa in colorless rhombs, m.p. 117～118°. *Anal.* Calcd. for $C_{13}H_{12}O_3$: 72.21; H, 5.59. Found : C, 72.05; H, 5.43. IR ν_{max}^{Nujol} cm^{-1} : 3340 (OH), 1675 (αβ-unsaturated C=O), 1660 (C=C).

The 2,4-dinitrophenylhydrazone prepared in the usual way was crystallized from dioxane in yellow fine needles, m.p. 211～212°. *Anal.* Calcd. for $C_{19}H_{16}O_6N_4$: C, 57.57; H, 4.07; N, 14.14. Found : C, 57.46; H, 3.80; N, 14.36.

3-Acetoxygris-2′-en-4′-one prepared by acetylation of XIIa with AcCl-pyridine (0～3°, 10 hr.) was crystallized from EtOH in colorless needles, m.p. 106～107°. *Anal.* Calcd. for $C_{15}H_{14}O_4$: C, 69.75; H, 5.46. Found : C, 69.57; H, 5.07.

3-Hydroxygrisan-4′-one (XIIIa)——A solution of 200 mg. of XIIa in 25 ml. of EtOH was shaken with H_2 at room temperature and atmospheric pressure in the presence of 80 mg. of 10% Pd-C. About 1.2 moles of H_2 was absorbed during 20 min., and the absorption stopped.

The catalyst was filtered and the solvent was distilled *in vacuo*, to leave colorless viscous oil, which solidified on trituration with petr. benzin. Crystallization from diisopropyl ether afforded 130 mg. of XIIIa in colorless rhombs, m.p. 96～97°. *Anal.* Calcd. for $C_{13}H_{14}O_3$: C, 71.54; H, 6.47. Found : C, 71.83; H, 6.46. IR ν_{max}^{Nujol} cm^{-1} : 3380 (OH), 1720 (coumaranone C=O).

Grisan-3,4′-dione (XIVa)——To a solution of 350 mg. of XIIIa in 5 ml. of AcOH was added dorpwise a solution of 300 mg. of CrO_3 in 0.3 ml. of H_2O and 2 ml. of AcOH at 45～50°. After stirring for 1 hr. at the same temperature, the green solution was cooled, diluted with 30 ml. of H_2O, and extracted with AcOEt.

The extract was dried, then the solvent removed to leave colorless viscous liquid, which solidified on trituration with EtOH. Crystallization from Et_2O afforded 110 mg. of XIVa in colorless plates, m.p. 89～90°. *Anal.* Calcd. for $C_{13}H_{12}O_3$: C, 72.21; H, 5.59. Found : C, 72.32; H, 5.56. IR ν_{max}^{Nujol} cm^{-1}: 1730, 1715. UV λ_{max}^{MeOH} mμ (log ε) : 251 (4.02), 326 (3.71).

The mono 2,4-dinitrophenylhydrazone was crystallized from dimethylformamide in orange yellow fine needles, m.p. 224～226°. *Anal.* Calcd. for $C_{19}H_{16}O_6N_4$: C, 57.57; H, 4.07; N, 14.14. Found : C. 57.86; H, 4.03; N, 14.43.

2,2-Bis(2-hydroxycarbonylethyl)-3-coumaranone (XVII)——To a stirred solution of 19.0 g. of methyl acrylate and 13.4 g. of 3-coumaranone in 35 ml. of dioxane was added dropwise 2 ml. of triton B at 35～40°. The original clear solution was separated into two layers.

After stirring for 3.5 hr. at the same temperature, the mixture was acidified with 10% HCl then diluted with 100 ml. of H_2O. The separated oil was taken up in $CHCl_3$. The extract was washed with 10% NaOH solution, H_2O, and dried.

The solvent was distilled to leave yellowish viscous liquid. Distillation at 180～187°/0.7 mm. afforded 15.8 g. of 2,2-bis(2-methoxycarbonylethyl)-3-coumaranone (XVI). Twelve and three-tenth grams of this diester (XVI) was refluxed with 100 ml. of 10% NaOH for 6 hr. Resulted clear solution was acidified with 10% HCl to congo red and extracted with Et_2O for several times. The extract was dried, and evaporated to leave colorless viscous liquid, which was solidified on being kept overnight in a refrigerator.

Crystallization from AcOEt afforded 8.8 g. of XVII in colorless prisms. m.p. 136～137°. *Anal.* Calcd. for $C_{14}H_{14}O_6$: C, 60.43; H, 5.07. Found : C, 60.31; H, 4.98.

Grisan-3,4′-dione (XIVb) from XVII——A solution of 11.1 g. of XVII, 0.4 g, of KCN in 80 ml. of Ac_2O was refluxed for 15 hr. Then Ac_2O was distilled *in vacuo*, to leave black gummy residue, which was extracted with AcOEt. The extract was washed with 10% Na_2CO_3, H_2O, dried, and evaporated. The solidified residue was crystallized from Et_2O to give 7.1 g. of XIVb in colorless prisms, m.p. 88～89°.

3-Hydroxygris-3′-en-2′-one (XIIb)——This compound was prepared from XIb by the same operation as shown in XIa → XIIa. XIIb was crystallized from benzene in colorless needles, m.p. 142～143°. Yield: 87%. *Anal.* Calcd. for $C_{13}H_{12}O_3$: C, 72.21; H, 5.59. Found: C, 72.37; H, 5.53.

3-Acetoxygris-3′-en-2′-one prepared in the usual way (AcCl-pyridine) was crystallized from aq. MeOH in colorless needles, m.p. 114～115°. *Anal.* Calcd. for $C_{15}H_{14}O_4$: C, 69.75; H, 5.46. Found: C, 69.53; H, 5.41.

Gris-3′-en-dione (XIIIb)——To a stirred solution of 0.74 g. of XIIb in 10 ml. of AcOH was added dropwise a solution of 0.5 g. of CrO_3 in 1.5 ml. of H_2O and 5 ml. of AcOH at 35～40°. After stirring for 2 hr. at the same temperature, the resulting green solution was diluted with 100 ml. of H_2O to deposite crude crystals, which were collected, washed with H_2O and dried. Recrystallization from EtOH afforded 0.51 g. of XIIIb in colorless needles, m.p. 117～118°. *Anal.* Calcd. for $C_{13}H_{10}O_3$: C, 72.89; H, 4.71. Found: C, 73.24; H, 4.65. IR ν_{max}^{Nujol} cm^{-1}: 1720, 1680.

The mono-2,4-dinitrophenylhydrazone was crystallized from aqueous dioxane in yellow needles, m.p. 194°(decomp.). *Anal.* Calcd. for $C_{19}H_{14}O_6N_4$: C, 57.86; H, 3.58; N, 14.21. Found: C, 57.99; H, 3.75; N, 14.24.

Grisan-3,2′-dione (XIVb)——A solution of 420 mg. of XIIIb in 50 ml. of EtOH was shaken with H_2 at room temperature and atmospheric pressure in the presence of 200 mg. of 10% Pd-C. About 1.3 moles of H_2 was absorbed during 2 hr. and absorption stopped. Then the catalyst was removed and the solvent was distilled *in vacuo*. A slightly yellow viscous residue was solidified after 2 weeks standing in a refrigerator. Crystallization from Et_2O-petr. ether afforded 340 mg. of XIVb in colorless prisms, m.p. 81～82°. *Anal.* Calcd. for $C_{13}H_{12}O_3 \cdot H_2O$ (room temp., 24 hr.): C, 69.32; H, 5.82. Found: C, 69.58; H, 5.47. IR ν_{max}^{Nujol} cm^{-1}: 1730, 1710.

The mono-2,4-dinitrophenylhydrazone was crystallized from dimethyl formamide in orange yellow plates, m.p. 187～189°. *Anal.* Calcd. for $C_{19}H_{16}O_6N_4$: C, 57.57; H, 4.07; N, 14.14. Found: C, 57.78; H, 3.75; N, 14.28.

Benzofuran-5-pent-2-enoic Acid (XVIII)——A solution of 300 mg. of XIIb in 3 ml. of MeOH and 2 ml. of 10% HCl was warmed at 70～75° for 15 min. Soon the oil separated from the clear solution. Then the solvent was removed *in vacuo*, and the residue was taken up in Et_2O. The etherial layer was extracted with saturated $NaHCO_3$ solution for several times. The whole alkali layer was acidified with 10% HCl.

The separated crystals, after being kept over night in a refrigerator, were collected, washed with cold H_2O, and dried. Recrystallization from aq. MeOH afforded 130 mg. of XVIII in colorless leaflets, m.p. 91～92°. *Anal.* Calcd. for $C_{13}H_{12}O_3$: C, 72.21; H, 5.59. Found: C, 72.10; H, 5.58.

Benzofuran-5-pentanoic Acid (XIX)——A solution of 30 mg. of XVIII in 6 ml. of EtOH was shaken with H_2 at room temperature and atmospheric pressure in the presence of 10 mg. of 10% Pd-C. After absorptions of 3.8 ml. of H_2 in 20 min., the hydrogenation stopped. The catalyst was filtered and the solvent was removed to leave solid residue. Crystallization from EtOH afforded 19 mg. of XIX in colorless needles, m.p. 121～122°. *Anal.* Calcd. for $C_{13}H_{14}O_3$: C, 71.54; H, 6.47. Found: C, 71.27; H, 6.60. UV λ_{max}^{MeOH} mμ (log ε): 247 (4.18), 276 (3.65), 283 (3.65).

Ethyl δ-Oxo-benzofuranvaleroate (XXIII)——Six and four-tenth grams of freshly distilled salicylic aldehyde was converted into its Na salt in 30 ml. of abs EtOH containing 1.2 g. of Na. The EtOH was evaporated to dryness at 130°/30 mm. Hg for 2 hr. Then 20 ml. of anhyd. benzene was added, and to the benzene suspension of Na salt, with stirring, a solution of 13.0 g. of the bromoketone (XXII) in 30 ml. of anhyd. benzene was added.

After refluxing for 4 hr. the mixture was washed with 5% NaOH solution, H_2O, and dried over anhyd. K_2CO_3. Evaporation of the solvent gave 13.0 g. of solid mass, which was crystallized from petr. benzine to give 11.3 g. of XXIII in colorless plates, m.p. 55～56°. *Anal.* Calcd. for $C_{15}H_{16}O_4$: C, 69.21; H, 6.20. Found: C, 69.30; H, 5.91. UV λ_{max}^{MeOH} mμ (log ε): 225 (3.82), 293 (4.32). IR ν_{max}^{Nujol} cm^{-1}: 1740, 1680.

The semicarbazone was crystallized from EtOH in colorless plates, m.p. 138～139°. *Anal.* Calcd. for $C_{16}H_{19}O_4N_3$: C, 60.55; H, 6.07; N, 13.24. Found: C, 60.17; H, 5.80; N, 12.94.

Benzofuran-5-pentanoic Acid: By the Reduction of XXIII——A solution of 6.5 g. of XXIII, 5.0 g. of 80% $H_2NNH_2 \cdot H_2O$, in 15 ml. of diethylene glycol was heated at 100～120° for 20 min. After cooling, 7 g. of pulverized KOH was added and the whole, with stirring, heated gradually.

The temperature reached to 190° after 3 hr. and the H_2O, formed in the course of the reaction was distilled off. Stirring was continued for 2 hr. at 180～190°, during which time the evolution of N_2 ceased.

When the solution was cooled to 170～180°, the white crystals started to separate. After cooling, 100 ml. of H_2O was added to the suspension and the mixture was extracted with Et_2O. The Et_2O layer was discarded and the alkali layer was acidified with 10% HCl. The separated crystals were collected, washed with H_2O and recrystallized from EtOH to give 5.3 g. of XIX in colorless needles, m.p. 122～123°. *Anal.* Calcd. for $C_{13}H_{14}O_3$: C, 71.54; H, 6.47. Found: C, 71.47; H, 6.38.

This compound was identical with the one derived from the grisenone (XIIb) by mixed melting point determination and comparison of IR spectra.

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Summary

4-(*o*-Methoxymethoxybenzoyl)-1,3-cyclohexanedione, having a *β*-tricarbonyl system, was synthesized by the interaction of disodio-1,3-cyclohexanediene and methyl *o*-methoxymethoxybenzoate in liquid ammonia.

4-(*o*-Hydroxybenzoyl)-1,3-cyclohexanedione, having a hypothetical *β*-tetracarbonyl system, was also prepared. Grisan-2′,3,4′-trione yielded from the *β*-tetraketone by means of alkaline ferricyanide oxidation. The structures of its two isomeric methyl enol ethers were proved by deriving them to grisan-2′,3- and grisan-3,4′-dione.

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31. Makoto Hayashi, Reiko Uchida, Tsutomu Unemoto, and Komei Miyaki : Enzymic Oxidation of Ethanolamine by Beef Serum.*1

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It has been already informed that there is an oxidase capable of oxidizing spermine, spermidine, several aliphatic and aromatic amines, and normal long-chain diamines in the sera of sheep, beef and goat.[1,2] Tabor, *et al.*,[2] using partially purified enzyme, elucidated the decomposition of primary monoamines and long-chain diamines. Yamada and Yasunobu[3] obtained an oxidase in the beef serum in a crystalline form.

One of authors[4] has already reported on the oxidation of polyamines, and during the process of this study it was found that the serum of beef or goat oxidatively decomposes alkanolamine, especially ethanolamine, besides afore-mentioned amines. Therefore, with the object of investigating whether the oxidase already reported and the ethanolamine oxidizing enzyme are identical or not, separation by DEAE-cellulose column chromatography was attempted after fractionation with ammonium sulfate. However, it was found that these oxidases could not be separated by the present method. The present paper describes the oxidation of ethanolamine by partially purified enzyme in beef serum.

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