Studies on Active Principles of Tars. X. The Structures and Some Reactions of Antifungal Constituents in *Pix Pini*[1)]

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By following the antifungal activity, four antifungal principles, acetovanillone (1), two new cyclic β-diketones, (2 and 3), and an unknown compound (4), were isolated from wood tar, *Pix Pini*, which has been used traditionally for the treatment of fungal diseases in Japan. The structures of 2 and 3 were established by synthesis to be 1,1′,3,3′-tetraoxo-2,2′-bicyclopentyl and its 4-methyl derivative, respectively. Chemical reactivities and physical properties of 2 and 3 are also described.

Keywords antifungal principle; wood tar; *Pix Pini*; acetovanillone; 1,1′,3,3′-tetraoxo-2,2′-bicyclopentyl; 4-methyl-1,1′3,3′-tetraoxo-2,2′-bicyclopentyl

As a part of our series of studies on the chemical elucidation of antifungal substances in tars,[1)] which have traditionally been used for treatment of fungal diseases in Japan, we have examined wood tar, *Pix Pini*. The known chemical constituents of *Pix Pini* include creosote, phenolic compounds,[2-7)] and others,[8-10)] but, to our knowledge, no pharmacological study has been conducted on the antifungal principles in the tar. In the present work, we have isolated four antifungal principles, a phenol derivative (**1**), two new cyclic β-diketones (**2** and **3**) and an unknown compound (**4**), determined the structures of **1**—**3** on the basis of spectral data, and confirmed them by synthesis. The results are presented here, together with some physicochemical data for **2** and **3**.

In the isolation process, the disk method with *Trichophyton interdigitale* was used to follow the antifungal activity of the material, as previously reported.[11)] Isolation of the active principles was achieved by suitable combinations of distillation, partition, steam-distillation and column chromatographies on silica gel. The procedures are summarized in Chart 1.

Pix Pini (1000 g)
| distilled under reduced pressure
active fraction I (bp 110—150 °C (4 mmHg)) (150 g) [8 mm]
| dissolved in petroleum ether (500 ml)
| and extracted with 85% phosphoric acid (40 ml × 5)
85% phosphoric acid fraction
| diluting with cold H_2O (1000 ml)
| and extracted with petroleum ether (60 ml × 5)
active fraction II (2.78 g) [10 mm]
| silica gel column chromatography with benzene, chloroform and ether
active fraction III (chloroform eluate) (1.04 g) [12—13 mm]
| 1) extracted with 5% NaOH (150 ml × 3)
| 2) neutralized with 5% HCl and extracted with ether (500 ml × 3)
active fraction IV (0.52 g) [15 mm]
| steam distilled
active fraction V (distillate) (0.26 g) [17—18 mm]
| silicic acid column chromatography with chloroform and recrystallization

active compounds

1 (15 mg) [10 mm]	**2** (15 mg) [33 mm]	**3** (7 mg) [25 mm]	**4** (2 mg) [9 mm]

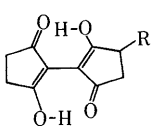
( ) indicates yield.
[ ] indicates diameter of inhibition zone (mm/0.5 mg).

Chart 1. Isolation of Antifungal Principles from *Pix Pini*

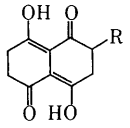
As shown in the chart, wood tar, *Pix Pini*, was fractionated by vacuum distillation into 6 fractions. The highest activity emerged in the fraction of bp 110—150 °C (4 mmHg) (active fraction I). This fraction was dissolved in petroleum ether and extracted into 85% phosphoric acid. The 85% phosphoric acid fraction was diluted with cold water, and extracted with petroleum ether, then benzene and finally ether. As the petroleum ether extract (active fraction II) showed the highest activity, it was subjected to silica gel column chromatography with benzene, chloroform and ether, successively. Firstly, we examined the chloroform eluate (active fraction III) because it showed higher antifungal activity than the other fractions. The acidic part (active fraction IV) of fraction III, obtained by extraction with aqueous sodium hydroxide, was steam-distilled to afford a pale yellowish oil (active fraction V). The distillate was subjected to column chromatography over silicic acid to afford four active compounds, **1**—**4**, each as colorless needles.

From the spectral data (nuclear magnetic resonance (NMR), infrared (IR), mass (MS) and ultraviolet (UV)), **1** was apparently identical with acetovanillone, 4-hydroxy-3-methoxyacetophenone. This was confirmed by direct comparison (mp, NMR, IR, MS and UV) with an authentic sample.[12)] The antifungal activity of the authentic sample was equal to that of the natural product.

It was suggested by comparison of spectral data of **2** with those of **3** that **3** is a methyl analog of **2**. Thus, these compounds were designated as uchinol and homouchinol. It was determined from MS and analytical data that the molecular formula of **2** is $C_{10}H_{10}O_4$. The ^1H- and ^{13}C-NMR and IR spectra of **2** suggest that the molecule is highly symmetrical, and it has two equivalent ethylene groups (2.60 ppm, s: $2\times-CH_2-CH_2-$) and two enolized α-substituted-β-diketone functions (ν_{max}^{KBr} 1550 cm^{-1}: HO–C=



I : R=H
II : R=CH_3



III
IV

C–C=O). From the above analyses, only the structure I or III seemed to be possible for **2** and II or IV for **3**.

We synthesized the possible structures by the following methods (Chart 2). Compounds I and II were obtained by the reactions of 2-bromocyclopentane-1,3-dione with cyclopentane-1,3-dione and 4-methylcyclopentane-1,3-dione,[13] respectively. Compounds III and IV were prepared by zinc reduction of the products prepared by Friedel–Crafts reaction of hydroquinone with succinic anhydride and 2-methylsuccinic acid, respectively.

The structures of **2** and **3** were determined as 1,1′,3,3′-tetraoxo-2,2′-bicyclopentyl (I) and 4-methyl-1,1′-3,3′-tetraoxo-2,2′-bicyclopentyl (II), respectively, by direct comparisons (mp, NMR, IR, MS and UV) with the synthetic samples. The antifungal activities of the synthetic compounds were equal to those of the natural products.

As the isolated compounds, **2** and **3**, have a novel 2,2′-bicyclopentyl skeleton with a 1,1′,3,3′-tetraone system, we were interested in their physicochemical properties. We firstly compared the physical properties of these compounds with those of 2-methylcyclopentane-1,3-dione.

The IR spectra of **2**, 2-methylcyclopentane-1,3-dione and their deuterated derivatives are presented in Fig. 1. Unambiguous assignment of the IR absorption bands was not possible, but certain conclusions can be reached. First, the absence of any strong absorption of **2** before 1800 cm^{-1} is not compatible with the enolic hydroxyl absorption of 2-methylcyclopentane-1,3-dione. Further, deuteration of **2** produces no marked change above 1800 cm^{-1}. From these observations we may infer that two exceptionally strong symmetric hydrogen bonds,[14] in which the hydrogen atoms are not sufficiently firmly bound to either oxygen atom to produce the characteristic enolic hydroxyl spectrum, exist in **2**. Support for this interpretation is given by the high symmetry of the ^1H- and ^{13}C-NMR spectra, as **2** has only two proton signals at 16.50 (2H, s) and 2.60 (8H, s) ppm and also 3 carbon signals at 201.7 (s), 110.2 (s), and 31.3 (t) ppm.

In addition, the absorption characteristics of **2** and **3** in the UV region are different from those of usual cyclic β-diketones, such as 2-methylcyclopentane-1,3-dione and cyclohexane-1,3-dione, which exist almost exclusively in enolic form even in a polar solvent. The enolic structure of 2-methylcyclopentane-1,3-dione shows strong absorption at 250 nm due to π–π^* transition in the *s*-trans enone system. On formation of the enolate ion in alkaline solution, the strong absorption at 250 nm shows a bathochromical shift to 268 nm.[15]

In contrast, in the case of **2**, which is completely enolized in the solvent, a slight hypsochromic shift (2 nm) was observed, such that the strong absorption at 270 nm, assignable to π–π^* transition, is shifted at 268 nm on enolate formation. To our knowledge, there is no previous report regarding such a hypsochromic shift of β-diketones. It could be accounted for by assuming that the electronic structure of **2** is greatly changed by the exceptionally strong symmetrical intramolecular hydrogen bonds, as compared with the case of 2-methylcyclopentane-1,3-dione.

Consequently, the question arose of whether the molecular structure of **2** is a resonance-stabilized form with two symmetrical hydrogen bonds, I_C,[16] or tautomeric forms, I_A and I_B. So, for elucidation of the physicochemical properties of both compounds, we conducted several reactions, such as halogenations, etherification, esterification, condensations with amines and carbonyl reagents and so on.

The reaction of **2** with chlorinating agents (SO_2Cl_2 and NCS (*N*-chlorosuccinimide)) proceeded in moderate overall yields, as depicted in Chart 3, to afford 4-chloro-1,1′,3,3′-tetraoxo-2,2′-bicyclopentyl **5**. Bromination of **2** with bromine or NBS (*N*-bromosuccinimide) could be effected in benzene to give the 4-brominated product **6**.

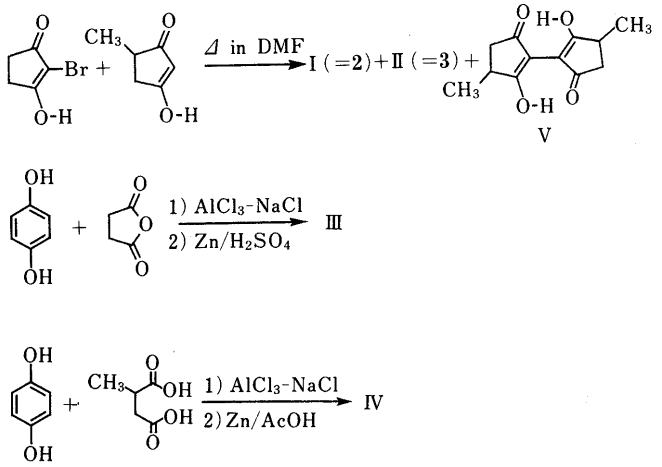
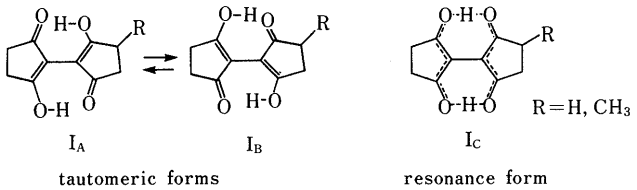


Chart 2. Preparations of I, II, III and IV



I_A I_B tautomeric forms I_C resonance form

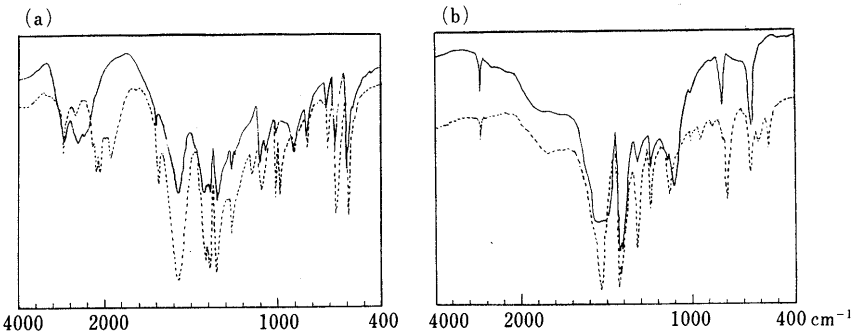


Fig. 1. The IR Spectra of **2** (a) and 2-Methylcyclopentane-1,3-dione (b) (Solid Lines) and the Deuterated Compounds (Broken Lines) (KBr Tablets)

But **2** does not provide any brominated derivative in the usual base-catalyzed bromination reaction, as used for the C-2 mono-bromination of carbonyl compounds. These results suggest that a free radical pathway leads to the 4-halogenated product.

We examined the chemical properties of the 4-halogenated compounds as follows. Solvolytic and base-induced eliminations on both halogenated compounds to yield compound VI did not proceed with aqueous potassium hydroxide, sodium ethoxide and DBU (diazabicycloundecane). But, in this reaction, they afforded nucleophilically substituted products, **7** and **8**, in moderate yields. This result shows that the enolate of **2** is stable under basic conditions and the β-elimination reaction is unfavorable, presumably owing to the highly strained state of compound VI.

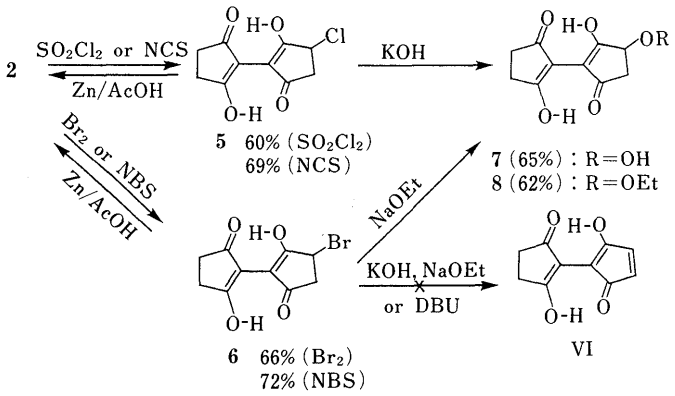


Chart 3

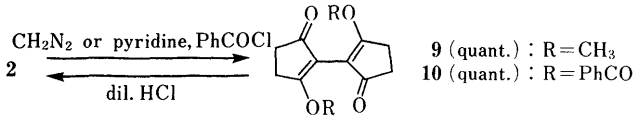
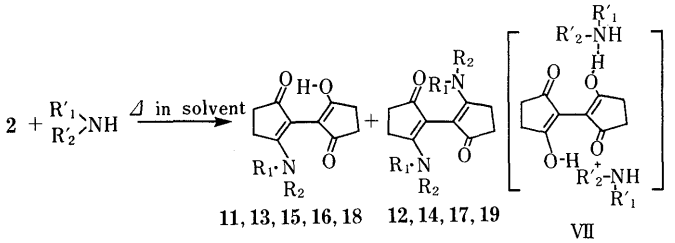


Chart 4



entry	R'_1	R'_2	R_1	R_2		
1	*n*-hexyl	H	*n*-hexyl	H	**11** (86%)	**12** (12%)
2	phenyl	H	phenyl	H	**13** (53%)	**14** (9%)
3	OH	H	OH	H	**15** (85%)	
4	NH_2	H	$N=C(CH_3)_2$	H	**16** (55%)	**17** (19%)
5	NH-CO-pyridyl	H	NH-CO-pyridyl	H	**18** (46%)	
6	$-CH_2-CH_2-CH_2-CH_2-$		$-CH_2-CH_2-CH_2-CH_2-$			**19** (quant.)

Chart 5

Etherification and acylation of **2** are illustrated in Chart 4. Diazomethane treatment of **2** gave the enol etherified compound **9** in quantitative yield. This product is very unstable even to diluted acid and base. Acylation of **2** with benzoyl chloride was accomplished to form the more stable enol ester **10** quantitatively in the presence of pyridine. In contrast, the base catalyzed acetylation of **2** with acetic anhydride or acetyl chloride by the use of pyridine, sodium acetate or boron trifluoride was not successful. The reason for this may be the stability of the enol ester produced, because the benzoyl ester **7** was readily hydrolyzed with dilute acid and base at room temperature in a short time.

Primary amines [10 eq (entries 1 and 2); 3 eq (entries 3 and 4)] and isonicotinic acid hydrazide (3 eq) also reacted with **2** to afford the corresponding condensed products, as shown in Chart 5, in good overall yields. The reaction of **2** with the secondary amine pyrrolidine (4 eq) gave **19** quantitatively. The condensed product obtained by reaction of **2** with amines was not hydrolyzed with hydrochloric acid; it takes the amineform, as confirmed by ^{1}H-NMR deuteration and decoupling experiments.

As judged from the yields of the condensed products (entries 1 and 2, and entries 4 and 5) in Chart 5, the basicity of the reagents may be reflected in the yields of the reaction products. It was observed by ^{1}H-NMR that the enolic proton signal (16.40 ppm, sharp) is broadened and shifted to higher field by adding pyridine to the NMR tube. Thus, we assumed that the intermediate salt, such as VII in Chart 5, formed by **2** and the reagent in the course of the reaction, reacts with the same reagent to afford the condensed product.

Heating of **2** with zinc gave naphthalene, while **3** gave naphthalene and 2-methylnaphthalene. This result is very interesting, because of the skeletal rearrangement, which may occur *via* the pathway illustrated in Chart 6.

In summary, we have isolated two new compounds, 1,1',3,3'-tetraoxo-2,2'-bicyclopentyl and 4-methyl-1,1',3,3'-tetraoxo-2,2'-bicyclopentyl, together with acetovanillone and an unknown compound as antifungal principles from wood tar, *Pix Pini*. It was found that these compounds are completely enolized and have two exceptionally strong intramolecular hydrogen bonds in the molecule. They show the expected reactivities due to their enol and carbonyl functional groups, and they also undergo an unique skeletal rearrangement, probably due to the unusual π-electron delocalized system, under the reaction conditions examined.

The above results leave open the question of the molecular structures of **2** and **3** in the ground state. The results of a detailed study of **2** and its derivatives made by using both spectroscopic and crystallographic methods will be reported elsewhere. In view of the unique skeleton and antifungal activity of these compounds, we are attempting

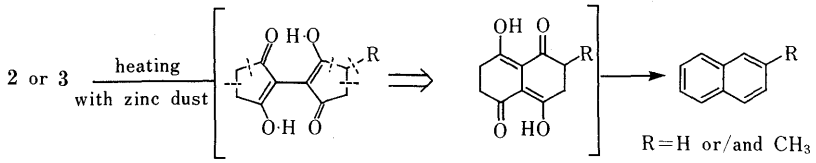


Chart 6

to develope a novel and convenient synthesis.

Experimental

Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected. UV and IR spectra were taken on a Shimadzu UV-360 spectrometer and a JASCO IRA-2 grating IR spectrometer, respectively. Low-resolution MS were recorded on Hitachi M-80A, JEOL JMS-AX505W and JEOL JMS D-100 instruments. NMR spectra were recorded on JEOL FX-90 and JEOL GX-270 spectrometers using tetramethylsilane (TMS) as an internal standard. For column chromatography, Kieselgel 60 and silica gel (70—230 mesh, Merck) were used.

Assay The disk method using *Trichophyton interdigitale* was employed for determination of the antifungal activity of the material, as reported previously.[11]

Material The wood tar, *Pix Pini*, used in this study was in accordance with the specification in JP VII.

Isolation of Antifungal Principles *Pix Pini* (1000 g) was distilled under reduced pressure at 3—4 mmHg to obtain the active fraction I, bp 115—150 °C (4 mmHg). This fraction (150 g) was dissolved in petroleum ether (500 ml), and the solution was extracted with 85% phosphoric acid (40 ml × 5). After dilution of the combined 85% phosphoric acid fraction with 5 volumes of H_2O, the solution was extracted with petroleum ether (60 ml × 5). The petroleum ether extract (2.78 g) was subjected to silica gel column chromatography with benzene, then chloroform and finally ether. The chloroform eluate (1.04 g) was dissolved in ether (100 ml) and then extracted with aqueous 5% NaOH (150 ml × 3). The extract was neutralized with 5% HCl, and extracted with ether (300 ml × 5). The acidic fraction thus obtained (0.52 g) was subjected to steam-distillation, and the distillate (2000 ml) was saturated with sodium chloride, then extracted with chloroform (500 ml × 5). The extract (0.26 g) was chromatographed over silicic acid (100 g) to yield four antifungal principles, **1** (15 mg), **2** (15 mg), **3** (7 mg) and **4** (2 mg), each as a colorless powder. Recrystallization of the active principle **1** from H_2O afforded colorless needles. The active compound **2** was recrystallized from *n*-hexane and **3** from ethanol to give colorless needles in each case.

1: Colorless needles, mp 111 °C, MS *m/z*: 166. $C_9H_{10}O_3$. ^{1}H-NMR (in $CDCl_3$) δ: 7.45—7.60 (2H, m), 7.30 (1H, s), 6.91 (1H, d, J=7.5 Hz), 6.15 (1H, s), 3.92 (3H, s), 2.55 (3H, s). IR ν_{max}^{KBr} cm^{-1}: 3300, 3050, 2950, 1660, 1570, 1500, 1395, 1215, 1195, 1030, 850. UV λ_{max}^{MeOH} nm (log ε): 230 (4.17), 277 (3.99), 305 (3.93).

2: Colorless needles, mp 211 °C. MS *m/z*: 194, 166, 138, 124, 110. *Anal.* Calcd for $C_{10}H_{10}O_4$: C, 61.86; H, 5.15. Found: C, 61.85; H, 5.23. ^{1}H-NMR (in $CDCl_3$) δ: 16.50 (2H, s), 2.60 (8H, s). ^{13}C-NMR (in $CDCl_3$) δ: 201.7 (s), 110.2 (s), 31.3 (t). IR ν_{max}^{KBr} cm^{-1}: 2950, 1550, 1420, 1410, 1320, 1245, 1140, 1105, 825, 560. UV λ_{max}^{MeOH} nm (log ε): 270 (4.29). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 268 (4.56).

3: Colorless needles, mp 114 °C, MS *m/z*: 208, 190, 180, 164, 152, 138, 124, 111. *Anal.* Calcd for $C_{11}H_{12}O_4$: C, 63.46; H, 5.77. Found: C, 63.76; H, 5.76. ^{1}H-NMR (in $CDCl_3$) δ: 16.77 (1H, s), 16.70 (1H, s), 2.86 (1H, dd, J=7.0, 18.5 Hz), 2.78 (1H, ddt, J=2.0, 7.0, 7.0 Hz), 2.64 (4H, s), 2.39 (1H, dd, J=2.0, 18.5 Hz), 1.26 (3H, d, J=7.0 Hz). ^{13}C-NMR (in $CDCl_3$) δ: 204.8 (s), 201.9 (s), 201.6 (s), 200.1 (s), 110.2 (s), 108.9 (s), 39.8 (t), 37.3 (d), 31.4 (t), 31.3 (t), 17.5 (q). IR ν_{max}^{KBr} cm^{-1}: 2950, 1550, 1420, 1250, 1100. UV λ_{max}^{MeOH} nm (log ε): 270 (4.29). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 267 (4.54).

Preparation of 2 A solution of cyclopentane-1,3-dione (4.80 g, 0.05 mol) and 2-bromocyclopentane-1,3-dione (8.8 g, 0.05 mol) in 50 ml of dimethylformamide was heated under reflux for 5 h. Removal of the solvent under reduced pressure gave a residue, which was subjected to silica gel column chromatography with chloroform to yield 970 mg of **2**. This was recrystallized from ethanol to afford colorless needles, mp 211.5 °C.

Preparation of 3 4-Methylcyclopentane-1,3-dione (5.5 g, 0.05 mol) was reacted with 2-bromocyclopentane-1,3-dione (8.8 g, 0.05 mol) in 50 ml of dimethylformamide (DMF) at 130 °C for 5 h and work-up as in the above experiment gave 194 mg of **2** and 916 mg of **3** as a colorless powder.[13] Compound **3** was recrystallized from *n*-hexane to afford colorless needles (mp 114 °C), and 24 mg of 4,4′-dimethyl-1,1′,3,3′-tetraoxo-2,2′-bicyclopentyl (V).

V: Colorless needles, mp 112 °C, MS *m/z*: 222, 204, 194, 166. *Anal.* Calcd for $C_{12}H_{14}O_4$: C, 64.86; H, 6.31. Found: C, 64.89; H, 6.28. ^{1}H-NMR (in $CDCl_3$) δ: 16.69 (0.7H, s), 16.61 (1H, s), 16.52 (0.3H, s), 1.80—3.05 (6H, m), 1.26 (3H, d, J=7.0 Hz). IR ν_{max}^{KBr} cm^{-1}: 2950, 1540, 1410, 1240, 1100. UV λ_{max}^{MeOH} nm (log ε): 272 (4.31). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 269 (4.47).

Preparation of III Anhydrous aluminum chloride (150 g) and sodium chloride (30 g) were fused over a direct flame with shaking. To the resultant complex, hydroquinone (11 g) and succinic anhydride (10 g) were added with stirring at 140 °C. The stirred mixture was heated rapidly to 200 °C, held at that temperature for 2 min, then allowed to cool to room temperature and poured into 15% HCl (1500 ml). The crude product was extracted with chloroform (500 ml) and purified by silica gel column chromatography with benzene to yield 2.0 g of 1,2,3,4-tetrahydro-5,8-dihydroxy-1,4-dioxonaphthalene. Zinc dust (5.3 g) was added in portions to a solution of the above product (2 g) in benzene (55 ml) and 20% H_2SO_4 (55 ml) with stirring. When the red color of the reaction solution changed to yellow, the reaction was quenched. The crude product in the benzene layer was purified by sublimation to give 800 mg of III (pale yellow powder) under reduced pressure.

III: Pale yellow powder, mp 203 °C, MS *m/z*: 194. *Anal.* Calcd for $C_{10}H_{10}O_4$: C, 61.86; H, 5.15. Found: C, 61.81; H, 5.20. ^{1}H-NMR (in $CDCl_3$) δ: 14.5 (2H, br s), 2.62 (8H, s). IR ν_{max}^{KBr} cm^{-1}: 2970, 2930, 1610, 1425, 1360, 1230, 1190, 930, 890. UV λ_{max}^{MeOH} nm (log ε): 221 (4.05), 310 (4.11).

Preparation of IV Friedel–Crafts reaction of hydroquinone (11 g) with methylsuccinic acid (13.7 g) catalyzed by aluminum chloride–sodium chloride complex gave 1,2,3,4-tetrahydro-5,8-dihydroxy-6-methyl-1,4-dioxonaphthalene (2.5 g). Zinc dust (5.0 g) was added in portions to a solution of the above product (2.5 g) in 100 ml of glacial acetic acid with stirring. When the red color of the reaction solution changed to yellow, the reaction was quenched by filtering off the zinc. The filtrate was diluted with H_2O (500 ml) and the resulting precipitates were recrystallized from ethanol to give 1.5 g of IV as light purple needles.

IV: Light purple needles, mp 127 °C, MS *m/z*: 208. *Anal.* Calcd for $C_{11}H_{12}O_4$: C, 63.46; H, 5.77. Found: C, 63.56; H, 5.66. ^{1}H-NMR (in $CDCl_3$) δ: 14.50 (1H, s), 14.25 (1H, br s), 2.10—2.95 (3H, m), 2.65 (4H, s), 1.21 (1H, d, J=7.0 Hz). IR ν_{max}^{KBr} cm^{-1}: 2950, 1605, 1215, 1180, 995. UV λ_{max}^{MeOH} nm (log ε): 221 (4.01), 312 (4.10).

Preparation of *O*-Deuterated Derivatives of 2 and 2-Methylcyclopentane-1,3-dione *O*-Deuterated derivatives of the examined compounds were prepared by recrystallization from CH_3OD (99%, Merck). Their extents of deuteration were about 95%, as evaluated by proton NMR spectroscopy.

Reaction of 2 with NCS A solution of **2** (194 mg, 1 mmol) and NCS (124 mg, 1 mmol) in 50 ml of chloroform was refluxed for 2 h in the presence of a catalytic amount of benzoyl peroxide. The reaction solution was concentrated in a rotary evaporator, and the residue was subjected to silica gel column chromatography with chloroform to give 4-chloro-1,1′,3,3′-tetraoxo-2,2′-bicyclopentyl (**5**) (157 mg) (68.6%). Compound **5** was recrystallized from methanol to give colorless needles.

5: Colorless needles, mp 115 °C, MS *m/z*: 230, 228, 193, 167, 154, 137. *Anal.* Calcd for $C_{10}H_9ClO_4$: C, 52.63; H, 3.94; Cl, 15.79. Found: C, 52.50; H, 4.07; Cl, 15.44. ^{1}H-NMR (in $CDCl_3$) δ: 16.32 (1H, s), 16.10 (1H, s), 4.61 (H, dd, J=3.0, 6.8 Hz), 3.21 (1H, dd, J=6.8, 18.0 Hz), 2.81 (1H, dd, J=3.0, 18.0 Hz), 2.60 (4H, s); IR ν_{max}^{KBr} cm^{-1}: 2950, 2935, 1540, 1400, 1250, 1070, 720, 675. UV λ_{max}^{MeOH} nm (log ε): 273 (4.25). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 267 (4.61).

Reaction of 2 with Sulfuryl Chloride Reaction of **2** with a 4-fold excess of sulfuryl chloride in carbon tetrachloride afforded **5** (60.0%).

Reduction of 5 with Zinc Dust Heating of **5** with an excess of zinc dust in glacial acetic acid at 100 °C for 2 h gave **2** quantitatively.

Reaction of 2 with Bromine A solution of bromine (160 mg, 1 mmol) in 10 ml of benzene was added dropwise to a solution of **2** (194 mg, 1 mmol) in 20 ml of benzene at room temperature. The reaction solution was stirred for 2 h at this temperature, then concentrated in a rotary evaporator under reduced pressure. The crude product was purified by silica gel column chromatography with chloroform to afford 4-bromo-1,1′,3,3′-tetraoxo-2,2′-bicyclopentyl (**6**) (187 mg) (66.1%). The product **6** was recrystallized from methanol, yielding fine colorless prisms.

6: Colorless prisms, mp 197 °C (dec.), MS *m/z*: 284, 282, 256, 254, 193, 165, 163, 148. *Anal.* Calcd for $C_{10}H_9BrO_4$: C, 42.40; H, 3.18; Br, 28.27. Found: C, 42.52; H, 3.18; Br, 28.41. ^{1}H-NMR (in $CDCl_3$) δ: 16.50 (1H, s), 16.25 (1H, s), 4.60 (1H, dd, J=3.0, 6.8 Hz), 3.25 (1H, dd, J=6.8, 18.0 Hz), 2.78 (1H, dd, J=3.0, 18.0 Hz), 2.60 (4H, s). IR ν_{max}^{KBr} cm^{-1}: 2950, 1540, 1420, 1403, 1370, 1070, 940, 675. UV λ_{max}^{MeOH} nm (log ε): 273 (4.25). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 267 (4.61).

Reaction of 2 with NBS Reaction of **2** with an equimolar amount of NBS in chloroform gave **6** (72.4%).

Reduction of 6 with Zinc Dust and Glacial AcOH Reduction of **6**

with zinc dust and glacial acetic acid in the manner described above afforded **2** quantitatively.

Reaction of 5 with Potassium Hydroxide A solution of **5** (115 mg, 0.5 mmol) in 10 ml of 20% aqueous potassium hydroxide was heated for 2 h at 100 °C. The reaction solution was neutralized with 10% HCl, then the crude product was extracted and purified by silica gel column chromatography with chloroform and methanol. 4-Hydroxy-1,1′,3,3′-tetraoxo-2,2′-bicyclopentyl (**7**) was recrystallized from methanol to afford colorless needles (53 mg, 65%).

7: Colorless needles, mp 170°C. MS *m/z*: 210, 192, 164, 138, 136. *Anal.* Calcd for $C_{10}H_{10}O_5$: C, 57.14; H, 4.76. Found: C, 57.19; H, 4.74. ^{1}H-NMR (in $CDCl_3$) δ: 16.25 (2H, s), 4.60 (1H, dd, J=3.0, 6.0 Hz), 2.98 (1H, dd, J=6.0, 18.0 Hz), 2.63 (4H, s), 2.43 (1H, dd, J=3.0, 18.0 Hz). IR ν_{max}^{KBr} cm^{-1}: 3400, 1500, 1400, 1360, 1260, 1090, 1065. UV λ_{max}^{MeOH} nm (log ε): 271 (4.21). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 268 (4.48).

Reaction of 5 with Sodium Ethoxide Reaction of **5** with sodium ethoxide gave 4-ethoxy-1,1′,3,3′-tetraoxo-2,2′-bicyclopentyl (**8**) in 62% yield as a colorless powder. Compound **8** was recrystallized from ethanol to give colorless needles.

8: Colorless needles, mp 113 °C. MS *m/z*: 238, 193, 175, 165, 137, 110. *Anal.* Calcd for $C_{12}H_{14}O_5$: C, 60.50; H, 5.88. Found: C, 60.71; H, 5.76. ^{1}H-NMR (in $CDCl_3$) δ: 16.30 (1H, s), 16.10 (1H, s), 4.29 (1H, dd, J=3.0, 6.0 Hz), 3.62 (2H, q, J=6.4 Hz), 2.94 (1H, dd, J=6.0, 18.0 Hz), 2.62 (4H, s), 2.50 (1H, dd, J=3.0, 18.0 Hz), 1.24 (3H, t, J=6.4 Hz). IR ν_{max}^{KBr} cm^{-1}: 2950, 1540, 1420, 1400, 1240, 1100, 820, 560. UV λ_{max}^{MeOH} nm (log ε): 272 (4.23). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 268 (4.46).

Reaction of 2 with Diazomethane Compound **2** (194 mg, 1 mmol) was allowed to react with diazomethane (420 mg, 10 mmol) in 50 ml of methanol at 3 °C for 1 h. The reaction mixture was concentrated in a rotary evaporator under reduced pressure, and the residue was recrystallized from acetone to afford colorless plates (220 mg, 99.5%) of 1,1′-dimethoxy-3,3′-dioxo-2,2′-bicyclopentenyl (**9**).

9: Colorless plates, mp 229 °C, MS *m/z*: 222, 207, 194, 192, 180, 152. *Anal.* Calcd for $C_{12}H_{14}O_4$: C, 64.86; H, 6.35. Found: C, 64.89; H, 6.39. ^{1}H-NMR (in $CDCl_3$) δ: 3.95 (6H, s), 2.20—2.90 (8H, m). IR ν_{max}^{KBr} cm^{-1}: 2950, 1676, 1580, 1345, 1284, 1042, 920, 596. UV λ_{max}^{MeOH} nm (log ε): 254 (4.44). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 260 (4.44).

Hydrolysis of 9 with Diluted Hydrochloric Acid Compound **9** was hydrolyzed immediately with diluted HCl to afford **2** quantitatively at room temperature.

Reaction of 2 with Benzoyl Chloride Benzoyl chloride (560 mg, 4 mmol) was added dropwise to a solution of **2** (194 mg, 1 mmol) in 4 ml of pyridine at room temperature. After standing for 1 h, the resulting mixture was concentrated to dryness in a rotary evaporator. The crude product was purified by silica gel column chromatography with chloroform and recrystallized from ethanol to afford colorless plates (400 mg, 99.5%) of 1,1′-dibenzoyloxy-3,3′-dioxo-2,2′-bicyclopentenyl (**10**).

10: Colorless plates, mp 173 °C, MS *m/z*: 194, 149, 122, 105. *Anal.* Calcd for $C_{24}H_{18}O_6$: C, 71.64; H, 4.48. Found: C, 71.83; H, 4.49. ^{1}H-NMR (in $CDCl_3$) δ: 7.97 (4H, d, J=3.0, 8.1 Hz), 7.25—7.60 (6H, m), 2.97—3.30 (4H, m), 2.45—2.76 (4H, m). IR ν_{max}^{KBr} cm^{-1}: 3025, 2970, 1750, 1687, 1597, 1225, 1050, 718. UV λ_{max}^{MeOH} nm (log ε): 240 (4.49). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 230 (4.55), 266 (4.53).

Hydrolysis of 10 with Diluted Hydrochloric Acid Compound **10** was hydrolyzed immediately with diluted hydrochloric acid to afford **2** quantitatively at room temperature.

Reaction of 2 with *n*-Hexylamine A solution of **2** (194 mg, 1 mmol) and *n*-hexylamine (600 mg, 10 mmol) in 50 ml of ethanol was allowed to heat for 4 h under reflux. The resulting mixture was concentrated to dryness in a rotary evaporator, then the resultant residue was chromatographed over silica gel with chloroform to give 1-*n*-hexylamino-1′-hydroxy-3,3′-dioxo-2,2′-bicyclopentenyl (**11**) (238 mg, 86%) and 1,1′-di-*n*-hexylamino-3,3′-dioxo-2,2′-bicyclopentenyl (**12**) (44 mg, 12%), each as a colorless powder. Both products were recrystallized from ethanol to afford colorless needles, respectively.

11: Colorless needles, mp 79 °C, MS *m/z*: 277, 219, 205, 192, 178, 164. *Anal.* Calcd for $C_{16}H_{23}NO_3$: C, 69.31; H, 8.30; N, 5.05. Found: C, 69.49; H, 8.34; N, 5.13. ^{1}H-NMR (in $CDCl_3$) δ: 17.40 (1H, s), 11.50 (1H, br s), 3.24 (2H, dt, J=6.0, 6.0 Hz), 2.30—2.95 (4H, m), 2.48 (4H, s), 1.10—2.05 (8H, m), 0.90 (3H, t, J=6.1 Hz). IR ν_{max}^{KBr} cm^{-1}: 2920, 2850, 1640, 1540, 1420, 1305. UV λ_{max}^{MeOH} nm (log ε): 288 (4.36). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 268 (4.44), 285 (4.42).

12: Colorless needles, mp 102 °C, MS *m/z*: 360, 345, 307. *Anal.* Calcd for $C_{22}H_{36}N_2O_2$: C, 73.33; H, 10.00; N, 7.78. Found: C, 73.29; H, 9.81; N, 7.54. ^{1}H-NMR (in $CDCl_3$) δ: 10.80 (2H, br s), 3.16 (4H, dt, J=6.0, 6.0 Hz), 2.63 (4H, s), 2.35 (4H, s), 1.10—1.92 (16H, m), 0.82 (6H, t, J=5.9 Hz). IR ν_{max}^{KBr} cm^{-1}: 2920, 1600, 1508, 1458. UV λ_{max}^{MeOH} nm (log ε): 257 (4.29), 307 (4.24). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 233 (4.16), 303 (4.54).

Reaction of 2 with Aniline Reaction of **2** (194 mg, 1 mmol) with aniline (1.00 g, 10 mmol), as in the above experiment, gave 1-anilino-1′,3,3′-trioxo-2,2′-bicyclopentenyl (**13**) (143 mg, 53%) and 1,1′-dianilino-3,3′-dioxo-2,2′-bicyclopentenyl (**14**) (30 mg, 9%), each as a light yellow powder. Each compound was recrystallized from ethanol to afford light yellow prisms.

13: Light yellow prisms, mp 175 °C, MS *m/z*: 269, 193, 184. *Anal.* Calcd for $C_{16}H_{15}NO_3$: C, 71.38; H, 5.58; N, 5.20. Found: C, 71.61; H, 5.58; N, 5.08. ^{1}H-NMR (in $CDCl_3$) δ: 17.00 (1H, br), 13.15 (1H, br s), 7.15—7.46 (5H, m), 2.34—2.90 (4H, m), 2.52 (4H, s). IR ν_{max}^{KBr} cm^{-1}: 2940, 2450 (br), 1680, 1540, 1490, 1480, 1465, 1422, 1380, 1340, 1305, 760, 550. UV λ_{max}^{MeOH} nm (log ε): 260 (4.21), 298 (4.16). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 265 (4.21), 320 (4.15).

14: Light yellow prisms, mp 224 °C, MS *m/z*: 344, 167, 149, 93. *Anal.* Calcd for $C_{22}H_{20}N_2O_2$: C, 76.74; H, 5.81; N, 8.14. Found: C, 76.50; H, 5.86; N, 7.90. ^{1}H-NMR (in $CDCl_3$); δ: 13.80 (2H, br s), 7.00—7.45 (10H, m), 2.90 (4H, s), 2.52 (4H, s). IR ν_{max}^{KBr} cm^{-1}: 2950, 2400 (br), 1630, 1600, 1580, 1500, 1380, 1300, 940, 765, 705. UV λ_{max}^{MeOH} nm (log ε): 260 (4.03), 298 (4.16). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 265 (4.03), 320 (4.15).

Reaction of 2 with Hydroxylamine Reaction of **2** (194 mg, 1 mmol) with hydroxylamine (99 mg, 3 mmol) in the same manner as described above gave 1-nitroso-1′-hydroxy-3,3′-dioxo-2,2′-bicyclopentenyl (**15**) (178 mg, 85%) (green powder). Compound **15** was recrystallized from ethanol to give dark green needles.

15: Dark green needles, mp 160 °C (dec.), MS *m/z*: 209, 194, 164, 135. *Anal.* Calcd for $C_{10}H_{11}NO_4$: C, 57.42; H, 5.26; N, 6.70. Found: C, 57.22; H, 5.29; N, 6.52. ^{1}H-NMR (in CD_3OD) δ: 2.70—3.00 (2H, m), 2.49 (4H, s), 2.30—2.62 (2H, m). IR ν_{max}^{KBr} cm^{-1}: 2950, 2600 (br), 1610, 1510, 1478, 1380, 1030. UV λ_{max}^{MeOH} nm (log ε): 286 (4.20). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 250 (4.18), 311 (4.15).

Reaction of 2 with Hydrazine A solution of **2** (194 mg, 1 mmol) and hydrazine monohydrate (150 mg, 3 mmol) in 50 ml of benzene was refluxed for 30 min, then 50 ml of acetone was added and the whole was further heated under reflux for 2 h. The reaction mixture was concentrated by rotary evaporation, and the residue was purified by silica gel column chromatography with chloroform, then recrystallized from methanol to give 1-isopropylidenehydrazono-1′,3,3′-trioxo-2,2′-bicyclopentyl (**16**) (124 mg, 55%) and 1,1′-disiopropylidenehydrazono-3,3′-dioxo-2,2′-bicyclopentenyl (**17**) (60 mg, 19%), each as light green needles.

16: Light green needles, mp 148 °C, MS *m/z*: 248, 192, 164. *Anal.* Calcd for $C_{13}H_{16}N_2O_3$: C, 62.90; H, 6.45; N, 11.29. Found: C, 62.74; H, 6.60; N, 11.45. ^{1}H-NMR (in $CDCl_3$) δ: 17.40 (1H, br s), 14.05 (1H, br s), 2.80—3.15 (2H, m), 2.18—2.65 (2H, m), 2.52 (4H, s), 2.08 (3H, s), 2.01 (3H, s). IR ν_{max}^{KBr} cm^{-1}: 3400 (br), 2975, 1540, 1425, 1338, 1315, 1120. UV λ_{max}^{MeOH} nm (log ε): 226 (4.16), 305 (4.26). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 256 (4.26), 323 (4.27).

17: Light green needles, mp 216 °C, MS *m/z*: 302, 248, 246, 192, 189, 176, 149. *Anal.* Calcd for $C_{16}H_{22}N_4O_2$: C, 63.58; H, 7.28; N, 18.54. Found: C, 63.60; H, 7.25; N, 18.41. ^{1}H-NMR (in $CDCl_3$) δ: 14.70 (2H, br s), 3.00 (4H, s), 2.45 (4H, s), 2.07 (6H, s), 2.00 (6H, s). IR ν_{max}^{KBr} cm^{-1}: 2940, 1625, 1595, 1500, 1440, 1425, 1300, 1080. UV λ_{max}^{MeOH} nm (log ε): 265 (4.37), 390 (4.50). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 254 (4.41), 333 (4.46). UV $\lambda_{max}^{1\,N\,HCl}$ nm (log ε): 262 (4.13), 358 (4.55).

Reaction of 2 with Isonicotinic Acid Hydrazide A solution of **2** (194 mg, 1 mmol) and isonicotinic acid hydrazide (414 mg, 3 mmol) in 40 ml of ethanol was heated for 4 h under reflux, then concentrated in a rotary evaporator. The residue was chromatographed over silica gel with chloroform and methanol to give 1-(isonicotinic acid hydrazono)-1′-hydroxy-3,3′-dioxo-2,2′-bicyclopentenyl (**18**) as a light yellow powder. Compound **18** was recrystallized from ethanol to give light brown needles (145 mg, 46%).

18: Light brown needles, mp 160—170 °C (dec.), MS *m/z*: 313, 285, 207. *Anal.* Calcd for $C_{16}H_{15}N_3O_4$: C, 61.34; H, 4.79; N, 13.42. Found: C, 61.04; H, 4.86; N, 13.60. ^{1}H-NMR (in CD_3OD) δ: 8.60 (2H, dd, J=1.8, 6.0 Hz), 7.20 (2H, dd, J=1.8, 6.0 Hz), 2.55—2.90 (2H, m), 2.52 (4H, s), 2.30—2.55 (2H, m). IR ν_{max}^{KBr} cm^{-1}: 3200, 1642, 1507, 1405, 1305. UV λ_{max}^{MeOH} nm (log ε): 282 (4.29). UV $\lambda_{max}^{1\,N\,NaOH}$ nm (log ε): 262 (4.31), 385 (4.32).

Reaction of 2 with Pyrrolizine A solution of **2** (194 mg, 1 mmol) and pyrrolizine (284 mg, 4 mmol) in 50 ml of benzene was heated azeotropically under reflux for 2 h. After removal of the solvent under reduced

pressure, the crude product was purified by silica gel column chromatography with chloroform and methanol to yield 1,1′-bi-(*N*-pyrrolizyl)-3,3′-dioxo-2,2′-bicyclopentenyl (**19**) as an oily material, which was recrystallized as the picrate from ethanol to afford yellow plates (590 mg, 99%).

Picrate of **19**: Yellow plates, mp 231 °C. MS *m/z*: 300, 283, 255, 229, 191, 149. *Anal.* Calcd for $C_{18}H_{24}N_2O_2 \cdot C_6H_3N_3O_7$: C, 54.44; H, 5.10; N, 13.23. Found: C, 54.27; H, 5.12; N, 13.59. ^{1}H-NMR (in $CDCl_3$) δ: 9.72 (1H, br s), 8.70 (2H, s), 3.30—3.72 (8H, m), 2.71 (4H, s), 2.58 (4H, s), 1.80—2.21 (8H, m). IR ν_{max}^{KBr} cm^{-1}: 3000 (br), 1635, 1600, 1560, 1520, 1340, 1300, 1264. UV λ_{max}^{MeOH} nm (log ε): 322 (4.45).

Reaction of 2 with Zinc Dust The mixture of **2** (6 mg, 0.03 mmol) and zinc dust (50 mg) was heated in a capillary tube with a direct flame, and the sublimed material was extracted with chloroform. Analysis of the products thus obtained by gas liquid chromatography (GLC) revealed the presence of naphthalene. The same reaction of **3** with zinc afforded naphthalene and 2-methylnaphthalene.

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