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STUDIES ON THE CONSTITUENTS OF UMBELLIFERAE PLANTS—X

STEREOCHEMISTRY OF 3-BUTYLHYDROPHTHALIDES—1

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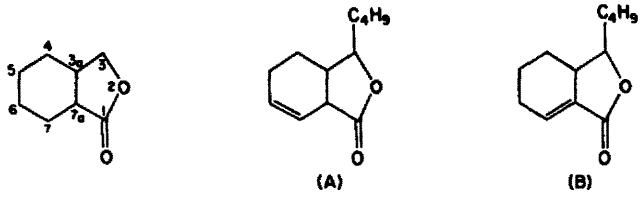
Abstract—The relative stereochemistry of cnidilide (I) and neocnidilide (II) has been elucidated by preparation and characterization of the racemates of the related compounds.

MITSUHASHI and MURAMATSU[1] have established that cnidilide (I) and neocnidilide (II), which are new 3-butylhydrophthalides isolated from *Cnidium officinale* Makino (Umbelliferae), have the structures, A and B, respectively, shown below without stereochemistry.



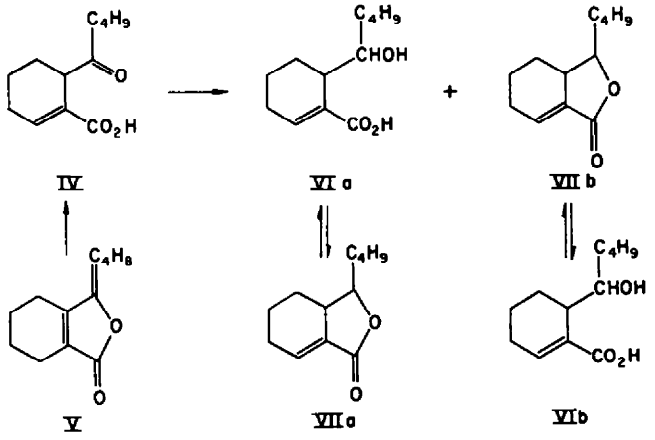
Noguchi[2] presented some evidence on the stereochemistry of these compounds from the same plant and from celery. This discussion does not appear useful because his samples are considered to be a mixture of related compounds; furthermore, the position of the double bond has been found to be incorrect, as noted in the previous paper.[1] We, therefore, start afresh.

The planar structure (B) is also assigned to isocnidilide (III), obtained by treatment of I with alcoholic potassium hydroxide.[1] B has two asymmetric carbon atoms; accordingly, four optically active isomers and two racemates can exist. Since the IR spectra of II and III are closely similar, but slightly different in the finger-print region, they are considered to be diastereomeric to each other.

Sedanonic acid (IV), prepared by hydrolysis of dihydroligustilide (V),[3] was reduced with sodium borohydride in water. Acidification of the reaction mixture afforded a crystalline hydroxy-acid (VIa) and an oily lactone (VIIb). Compound VIa gave an oily lactone (VIIa) by heating under reflux in toluene and VIIb gave a crystalline hydroxy-acid (VIb) by alkaline hydrolysis and subsequent acidification under cooling. Comparison of IR spectra of VIIa and VIIb indicates that they are diastereomeric to each other. Although this would be expected from their origin, the possibility that

[1] H. Mitsuhashi and T. Muramatsu, *Tetrahedron* **20**, 1971 (1964).
[2] T. Noguchi, *J. Pharm. Soc. Japan* **54**, 91 (1934).
[3] H. Mitsuhashi and U. Nagai, *Tetrahedron* **19**, 1277 (1963).

VIIa and VIIb might be the same substance must be considered. They are distinguished clearly, however, by comparison of their NMR spectra and the properties of the corresponding hydroxy-acids, VIa and VIb.



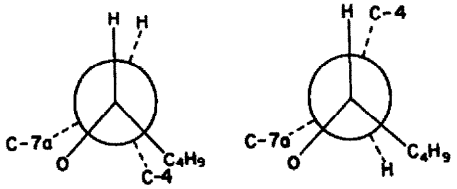
Since VIIa and VIIb are derived from V and so have no optical rotatory power, they represent the two possible racemates of the structure (B), and therefore the racemic forms of II and III. Careful comparison of their IR spectra shows that VIIa and III belong to the same series, and so do VIIb and II. Therefore, if it is determined whether VIIa or VIIb, is *cis* or *trans*, then the relative configurations of II and III will be known.

Inspection of the Dreiding molecular model of the structure (B) indicates the nearly eclipsed conformation of the substituents on the carbon atoms C-3 and C-3a, which is expected from the planarity of the five-membered ring due to the presence of an exocyclic double bond conjugated with the lactonic carbonyl group.

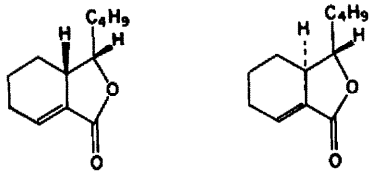
This situation makes the *cis*-isomer more strained than the *trans*-isomer by non-bonded interaction between the C-4-methylene and the butyl groups. Since a similar situation would exist in the transition state of lactonization of the corresponding hydroxy-acids, VIa and VIb, the hydroxy-acid, which lactonizes more readily, is considered to be *threo*, and the one, which resists lactonization, to be *erythro*. When IV was reduced with sodium borohydride, a mixture of VIa and VIIb was obtained. The result indicates that VIb lactonizes more readily than VIa, and this was confirmed by heating each separately under reflux in isopropyl ether. On the basis of these considerations, it is concluded that VIIa and III belong to the *cis*-, and VIIb and II to the *trans*-series.

Concerning the configuration of I, the structure (A) has three asymmetric carbon atoms, all of which are members of the lactone ring and each has one hydrogen atom. The relation between hydrogen atoms on carbon atoms C-3 and C-3a in I, is the same as in III, which has been established as *cis*. If the relation between the hydrogen atoms on C-3a and C-7a, i.e. the ring junction can be determined, the relative configuration of all asymmetric centres in I can be assigned.

Hydrogenation of I catalyzed with Pd-C in ethanol, yields quantitatively a crystalline saturated lactone (VIII[1], β-dihydrosedanolide, Noguchi[2]). Since the double



cis or *erythro* *trans* or *threo*



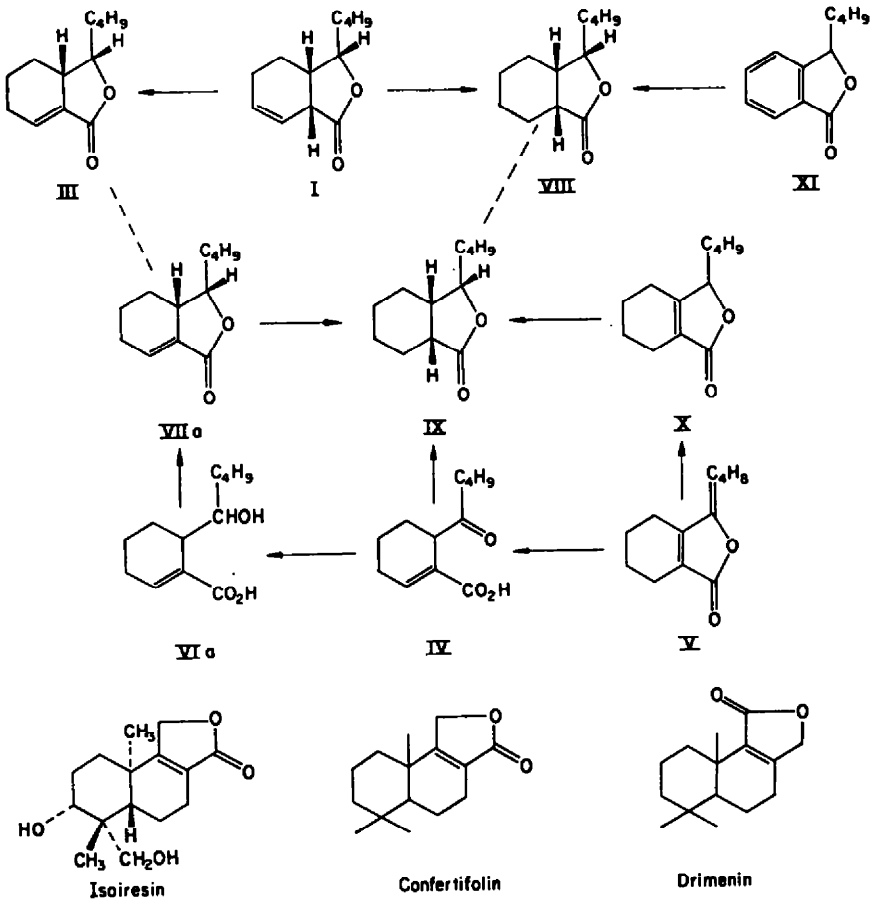
VII a and III VII b and II

bond of I is not concerned with the asymmetric centres of VIII, and as migration of the double bond during the hydrogenation is not probable under the neutral conditions, I is considered to have the same configuration as VIII. The racemate (IX) corresponding to VIII (IR comparison) was obtained by catalytic hydrogenation of the three compounds, IV, VIIa and tetrahydroligustilide (X). Noguchi prepared IX from IV and named it γ-dihydrosedanolide.[2] Barton and de Vries have prepared VIII by catalytic hydrogenation of (−)-3-butylphthalide (XI) and IX by the same route as Noguchi, and stated the relation between them.[4] The facts that IX is formed predominantly by catalytic hydrogenation of VIIa and X, and also that VIII is formed predominantly by catalytic hydrogenation of XI, indicate the *cis* ring junction in VIII and IX on the basis of the usual stereochemistry of catalytic hydrogenation[5,6] and the examples of such sesquiterpenoid lactones as isoiresin,[7] confertifolin, isodrimenin[8] etc.

This conclusion is also supported by the following results: Chromic acid oxidation of the hydroxy-acid (XII) corresponding to IX afforded a keto-acid (XIII), which was epimerized to an isomeric keto-acid (XIV) by alkaline treatment. The oily keto-acids, XIII and XIV, were characterized by formation of 2,4-dinitrophenylhydrazones, XV and XVI, respectively. The less stable keto-acid (XIII) is considered to be *cis*, and XIV to be *trans* from relative stability of 1,2-disubstituted cyclohexanes.

Attempts to convert IX into the corresponding *trans*-isomer under the influence of basic reagents such as potassium hydroxide and sodium methoxide in methanol, have failed, and the starting material was recovered. Although the results seem peculiar in contrast to the ready isomerization of dihydroconfertifolin to isodihydroconfertifolin,[8] it can be explained by assuming that the conformation with equatorial carboxyl group is more favoured than that with axial carboxyl in these molecules

[4] D. H. R. Barton and J. X. de Vries, *J. Chem. Soc.* 1916 (1963).
[5] S. Siegel and G. V. Smith, *J. Amer. Chem. Soc.* **82**, 6082 (1960); S. Siegel and M. Dunkel, *Adv. in Catalysis* **9**, 15 (1957).
[6] R. P. Linstead, W. E. Doering, Selby B. Davis, Philip Levine and R. R. Whetstone, *J. Amer. Chem. Soc.* **64**, 1985 (1942).
[7] Carl Djerassi and S. Burstein, *Tetrahedron* **7**, 37 (1959).
[8] H. H. Appel, J. D. Connoly, K. H. Overton and R. P. M. Bond, *J. Chem. Soc.* 4685 (1960).

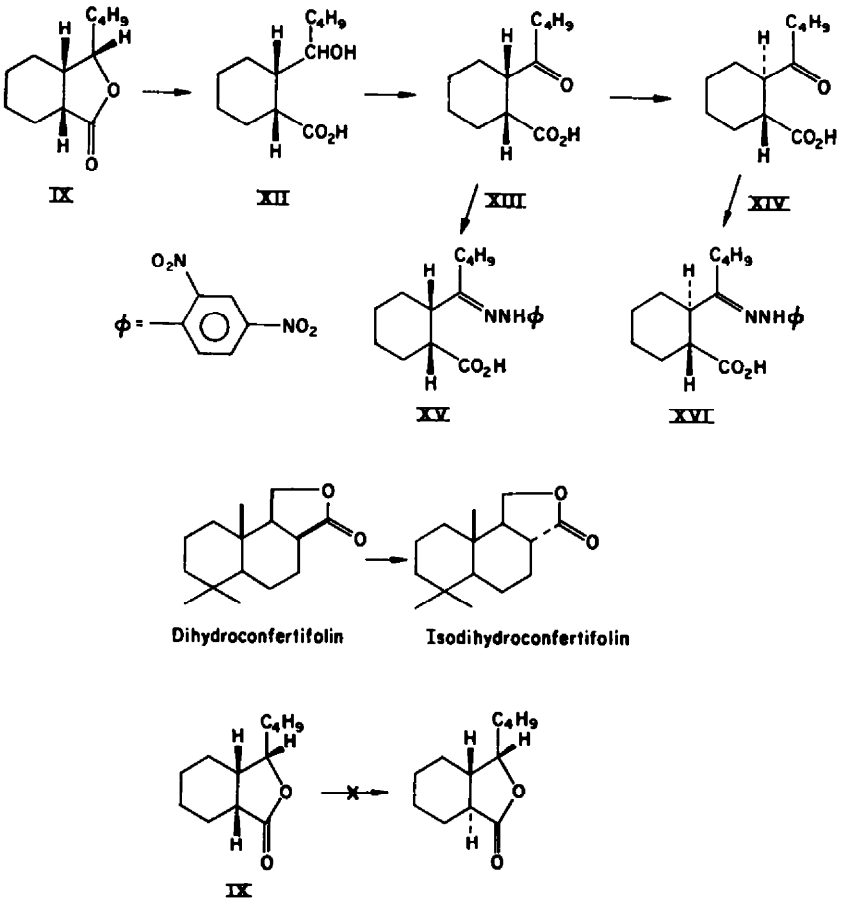


such as IX and XIII, and that the epimerization of XIII has occured only at the carbon atom adjacent to ketonic function. The latter assumption has been supported by high retention of rotatory power in the experiment with optically active compounds.[9]

The stereochemistry of I has now been assigned as that in which the hydrogen atoms attached to all three asymmetric carbon atoms are on the same side of the lactone ring, i.e. all *cis*. It has to be noted, however, that the conclusion is based on the probable assumption that the configuration will not change during hydrogenation of I to VIII. The *cis* ring junction of I itself is supported by the pattern shown by olefinic protons of I in its NMR spectrum.[1]

The fact that catalytic hydrogenation of VIIa, which has *cis* configuration at C-3 and C-3a afforded IX, and VIIb with *trans* configuration afforded a saturated lactone (XVII), which is clearly distinguished from IX, is consistent with the above conclusion, though a small amount of X is formed in both cases. The consideration that the catalyst will attack from the side not hindered by the butyl group at C-3 and that the all *cis* product will be given predominantly in hydrogenation of X and XI, also leads to the same conclusion.

[9] It will be described in the following paper on the absolute configuration of I.



EXPERIMENTAL

M.ps are uncorrected. UV spectra were measured with a Shimadzu recording spectrophotometer in EtOH solution. Column chromatography was done with silicic acid (100 mesh, Mallinckrodt) as adsorbent and $CHCl_3$ as eluent. NMR spectra were measured in CCl_4 and using tetramethylsilane as internal standard.

Reduction of sedanonic acid (IV) *with sodium borohydride*

(a) Sedanonic acid (1·5 g) was dissolved in water containing slight excess NaOH, mixed with an alkaline aqueous solution of $NaBH_4$ (0·7 g), and left in an incubator at 37° for 2 days. Acetone was added dropwise to the reaction mixture until evolution of H_2 ceased. Acidification of the solution yielded a crystalline precipitate (1·25 g), which was filtered off. The filtrate was extracted with ether. The ethereal solution was washed with dil. $NaHCO_3$aq and water, and dried (Na_2SO_4). Evaporation of ether gave a neutral oil (VIIb; 0·1 g). The filtered crystalline precipitate (1·25 g) was dissolved in ether and extracted with 5% $NaHCO_3$aq. Acidification of the alkaline aqueous solution with 10% HCl afforded the hydroxy-acid (VIa; 1·0 g), m.p. 115–117°.

(b) Sedanonic acid (2·0 g) was dissolved in water containing an equiv. amount NaOH. An aqueous solution of $NaBH_4$ (1·0 g) and a piece of NaOH was added to the solution of sedanonic acid, and left in an incubator at 37° for 2 days. Glacial acetic acid was added dropwise to the reaction mixture while stirring until generation of H_2 had ceased. The solution was poured into cold 10% HCl, and extracted with ether. The ethereal solution was washed with water, extracted with 5% $NaHCO_3$aq and dried (Na_2SO_4).

On evaporation of the solvent a neutral oil (0·6 g) was obtained. Acidification of the $NaHCO_3$aq layer with cold 10% HCl afforded a crystalline precipitate of the hydroxy-acid (VIa; 1·1 g). More VIa (0·4 g) was obtained from the filtrate by extraction with ether. Since the IR spectrum of the neutral oil indicated the presence of a carboxylic substance, its ethereal solution was washed with 5% $NaHCO_3$aq and water, and dried (Na_2SO_4). On evaporation of the solvent pure VIIb (0·13 g) was obtained.

The acid fraction (VIa) showed m.p. 114–115° (sint. at 108°), λ_{max} 215 mμ (log ε 3·97) in UV, and ν_{max}^{Nujol} 3450 (shoulder, O—H), 3300 (O—H), 2700–2400 (carboxyl O—H), 1685 (carboxyl C=O), 1640 (conjugated C=C) cm^{-1} in IR spectrum.

The neutral fraction (VIIb) showed $n_D^{19°}$ 1·4916, λ_{max} 216 mμ (log ε 3·52) in UV, $\nu_{max}^{CHCl_3}$ 1755 (γ-lactone C=O), 1682 (conjugated C=C) cm^{-1} in IR, and τ: 9·15 (3H), 6·40 (1H multiplet), 3·75 (1H doublet) in NMR spectrum. (Found: C, 74·32; H, 9·78. $C_{12}H_{16}O_2$ requires: C, 74·19; H, 9·34%).

Lactonization of the hydroxy-acid (VIa)

The hydroxy-acid (900 mg) was heated in toluene (30 ml) under reflux for 8·5 hr. The reaction mixture was diluted with ether and extracted with 5% $NaHCO_3$aq. The organic layer was washed with water and dried (Na_2SO_4). After removal of solvents, ether and toluene, the residue was distilled *in vacuo*, and a neutral oil (670 mg), b.p. $_{8}$145° was obtained. From the aqueous layer, the starting material (12 mg) was recovered. The neutral oil (VIIa) showed n_D^{21} 1·4960, λ_{max} 218 mμ (log ε 3·63) in UV, $\nu_{max}^{CHCl_3}$ 1751 (γ-lactone C=O), 1682 (conjugated C=C) cm^{-1} in IR, and τ: 9·15 (3H), 5·70 (1H multiplet), 3·73 (1H triplet) in NMR spectrum. (Found: C, 74·05; H, 9·16. $C_{12}H_{16}O_2$ requires: C, 74·19; H, 9·34%).

Hydrolysis of the lactone (VIIb).

The lactone (70 mg) and 5% methanolic NaOH were sealed in a test-tube, heated on a boiling water-bath for 1 hr. Water was added to the reaction mixture, and MeOH was evaporated under red. press. The resultant aqueous solution was washed with ether, acidified with 10% HCl carefully under cooling in an ice-salt bath, and extracted with cold ether. The ethereal solution was washed with cold water, dried (over $CaCl_2$ in a refrigerator for 1 hr), and evaporated under red. press. The concentrated solution of the residual oil in methylene chloride was diluted with hexane and cooled strongly in an ice-salt bath.

Crystals were formed by scratching the wall of the vessel and leaving in a refrigerator for 1/2 hr. Needles of m.p. 82–85° (Kofler; sint. at 76°), the first crop (15 mg), were obtained.

The second crop (5 mg), m.p. 81–84° was obtained from the mother liquor. The hydroxy-acid (VIb) showed λ_{max} 210 mμ (log ε 3·41) in UV, and ν_{max}^{Nujol} 3200 (broad, carboxyl O—H), 1665 (conjugated carboxyl C=O and C=C) cm^{-1} in IR spectrum.

Hydrolysis of the lactone (VIIa)

The lactone (300 mg) was hydrolyzed with 5% methanolic NaOH (2·5 ml) in a sealed tube under N_2 atm. by heating on a boiling water-bath for 1 hr. The reaction mixture was diluted with water, freed from MeOH by evaporating under red. press., washed with ether, and acidified with 10% HCl. Filtration of the crystalline precipitate afforded the hydroxy-acid (VIa), m.p. 116–117° (200 mg). It showed no depression on admixture with VIa, m.p. 114–115° formed directly from IV, mixed m.p. 114–116°.

A neutral oil (100 mg) was obtained from the ether used for washing the alkaline solution.

Rate comparison of lactonization of the hydroxy-acids, VIa *and* VIb

The solution of each hydroxy-acid, VIa (12·5 mg) or VIb (10·6 mg), in isopropyl ether was refluxed for 6 hr, diluted with ethyl ether, extracted with 5% $NaHCO_3$aq, washed with water, and dried (Na_2SO_4). On removal of the solvent, the lactonized neutral fraction, 1·4 mg from VIa and 2·3 mg from VIb, was obtained.

The yields for lactonization of VIa and VIb were 11% and 22%, respectively.

Catalytic hydrogenation of the lactone (VIIa)

PtO_2 (20 mg) was suspended in acetic acid (10 ml) and reduced with H_2. The lactone (370 mg) was dissolved in acetic acid (5 ml), added to the suspension of catalyst, and shaken with H_2.

Hydrogen (48 ml) at 23° and 756 mm Hg was absorbed during 70 min. After removal of catalyst and solvent, the residual oil was dissolved in ether, washed with 5% $NaHCO_3$aq and water, and dried (Na_2SO_4). The chromatography of the ethereal residue afforded IX (200 mg), X (20 mg), and a mixture of both (30 mg).

Compound IX showed m.p. 33–37° (Kofler) and $\nu_{max}^{CHCl_3}$ 1765 (γ-lactone C=O) cm^{-1} in IR spectrum. (Found: C, 73·37; H, 10·18. Calc. for $C_{12}H_{20}O_2$: C, 73·43; H, 10·27%). Compound X was identified by comparison of IR spectrum with the authentic sample.

Catalytic hydrogenation of tetrahydroligustilide (X)

PtO_2 (150 mg) was suspended in acetic acid (10 ml) and reduced with H_2. Tetrahydroligustilide (240 mg) was dissolved in EtOH (10 ml), added to the suspension of catalyst, and shaken with H_2.

Hydrogen (42 ml) was absorbed at 18° and 762 mm Hg. After removal of catalyst and solvent. the residual oil (226 mg) was freed from acidic substances by the usual procedure. The neutral oil (180 mg) was crystallized by seeding, and recrystallized from cold hexane (dissolved in hexane at room temp and cooled to ca. −20° in a bath of solid CO_2 and MeOH). The first crop: 33 mg, m.p. 38–39°; the second crop: 48 mg, m.p. 35–38°, and that from mother liquor: 76 mg of still lower m.p.; mixed m.p. of the first crop with that from VIIa: 37–39°.

Catalytic hydrogenation of sedanonic acid (IV)

PtO_2 (0·2 g) was suspended in acetic acid (5 ml) and reduced with H_2. Sedanonic acid (1·5 g) was dissolved in acetic acid (15 ml), added to the suspension of catalyst, and shaken with H_2.

Hydrogen (357 ml) was absorbed during 4 hr at 20° and 753 mm Hg.

After removal of catalyst and solvent, the residual oil was distilled *in vacuo*. The distillate, $b.p._4$ 142–148° (1·1 g) was obtained, crystallized by seeding, and recrystallized from cold hexane, yield 0·7 g, m.p. 37·5–39·5° (Kofler). The lactone obtained here showed a very similar IR spectrum to that of the above specimen.

Hydrolysis of the lactone (IX)

(a) The lactone (55 mg) and 25% KOHaq (0·3 ml) were heated in a sealed tube on a boiling water-bath for 1/2 hr. The reaction mixture was diluted with water, washed with ether, cooled to ca. 0°, acidified with 10% H_2SO_4 and extracted with cold ether. The ethereal solution was washed with water, treated with solid CO_2 to remove water as ice, decanted from ice, and evaporated under red. press. The residue was dissolved in a small amount of methylene chloride, diluted with hexane until no more turbidity was formed, and left in a refrigerator for 2 hr. Crystalline precipitates were filtered, and washed with hexane. Recrystallization from methylene chloride and hexane yielded the hydroxy-acid (XII), plates, m.p. 98–99°, which showed $\nu_{max}^{CHCl_3}$ 3500 (shoulder, O—H), 3200–2600 (broad, carboxyl O—H), 1700 (carboxyl C=O) cm^{-1} in IR spectrum.

(b) The lactone (45 mg) was dissolved in MeOH (1·5 ml) and heated with 5% methanolic KOH (1·5 ml) in a sealed tube for 1 hr on a boiling water-bath. The reaction mixture was diluted with water, freed from MeOH by evaporation under red. press., acidified with 10% HCl under cooling in an ice-salt bath, and extracted with cold ether. The ethereal solution was dried (over $CaCl_2$ in a refrigerator for 2 hr), and evaporated under red. press. The residue was dissolved in small amount of methylene chloride, diluted with hexane, and left in a refrigerator for 1 hr. Rectangular plates, m.p. 91–96° (sint. at 85°; Kofler) were obtained.

Formation of 2,4-*dinitrophenylhydrazones*, XV *and* XVI, *of the keto-acids from the lactone* (IX)

The lactone (600 mg) was refluxed with 5% methanolic KOH (6 ml) for 1 hr. The reaction mixture was diluted with water, freed from MeOH by evaporating under red. press., and acidified with 10% HCl under strong cooling. The precipitate thus formed was filtered off and washed with cold water. The filtered cake of the precipitate (ca. 800 mg without drying) was crushed and dissolved in glacial acetic acid (3 ml), mixed with CrO_3 (220 mg) in acetic acid and left in a refrigerator for 2·5 days. MeOH (0·3 ml) was added to the reaction mixture to decompose excess CrO_3. After 1 hr, the reddish colour due to the presence of CrO_3 had disappeared, and the solvent was evaporated under red. press. Water and ether were added to the residue and shaken vigorously. The ethereal layer was separated, washed with water, and dried (Na_2SO_4). The ethereal solution was divided into 2 portions of equal volume. The first portion was evaporated. The residue was dissolved in EtOH, treated with a solution of 2,4-dinitrophenylhydrazine in ethanolic H_3PO_4 by heating for 5 min

on a boiling water-bath, and left at room temp for several hr. The 2,4-dinitrophenylhydrazone (XV) was recrystallized from EtOH and afforded a yellowish powder, m.p. 160–162°. (Found: C, 55·62; H, 6·19; N, 14·30. $C_{18}H_{24}O_8N_4$ requires: C, 55·09; H, 6·17; N, 14·28%). The second portion of the above ethereal solution was evaporated. The residue was heated with a solution of NaOH (0·5 g) in water (10 ml) on a boiling water-bath for 1·5 hr. When cool, the reaction mixture was washed with ether, acidified with 10% HCl, and extracted with ether. The ethereal solution was washed with water, dried (Na_2SO_4), and evaporated. 2,4-dinitrophenylhydrazone (XVI) was formed from the residue by the same procedure as above and afforded crystalline needles, m.p. 145–147° by recrystallization from EtOH. (Found: C, 55·71; H, 6·43; N, 14·73. $C_{18}H_{24}O_8N_4$ requires: C, 55·09; H, 6·17; N, 14·28%). The two 2,4-dinitrophenylhydrazones showed a single spot at different positions (XV was higher than XVI) in 4 systems of T.L.C. (MeOH:C_6H_6 = 1:3 and n-BuOH saturated with water on Alumina G, Merck; MeOH:C_6H_6 = 3:97 and MeOH:$CHCl_3$ = 1:99 on Toshin-Layer G).

Attempts to convert IX *into the* trans-*isomer*

(a) A solution of the lactone (50 mg) and KOH (50 mg) in MeOH (1 ml) was left at room temp for 24 hr. The reaction mixture was poured onto cold 10% HCl and extracted with ether. The ethereal solution was washed with 5% $NaHCO_3$aq and water, and dried (Na_2SO_4). On removal of the solvents, 36 mg of oil was obtained.

(b) The lactone (50 mg) was dissolved in a solution of metallic Na (30 mg) in MeOH (1 ml), and left at room temp for 24 hr. The reaction mixture was poured onto ice-water and extracted with ether. The ethereal solution was washed with water and dried (Na_2SO_4). The alkaline aqueous layer was acidified with cold 10% HCl, and extracted with ether. The ethereal solution was treated similarly; 14 mg of oil was afforded from the neutral fraction and 29 mg from the acidic fraction.

(c) The lactone (61 mg) was dissolved in a solution of metallic Na (45 mg) and MeOH (1·5 ml), sealed in a tube, and heated on a boiling water-bath for 3 hr. The reaction mixture was poured onto cold 10% HCl, and extracted with ether. The ethereal solution was washed with 5% $NaHCO_3$aq and water, and dried (Na_2SO_4). On removal of the solvent, 31 mg of oil was yielded.

All these oils showed IR spectra very similar to that of the starting lactone in $CHCl_3$ solution.

Catalytic hydrogenation of the lactone (VIIb)

PtO_2 (10 mg) was reduced with H_2 in acetic acid (10 ml). The lactone (152 mg) was dissolved in acetic acid, added to the suspension of catalyst, and shaken with H_2; 18 ml H_2 was absorbed at 20° and 752 mm Hg during 1 hr. After removal of catalyst and solvent, the residue was dissolved in ether, washed with 5% $NaHCO_3$aq and water, and dried (Na_2SO_4). On evaporation of ether, the oily residue (124 mg) was purified by chromatography.

The lactone (XVII; 90 mg) and X (10 mg) were obtained: XVII showed n_D^{20} 1·5288, b.p.$_{5\cdot5}$158° (micro Emich), and $\nu_{max}^{CHCl_3}$ 1770 (γ-lactone C=O) cm^{-1} in IR spectrum. (Found: C, 73·17; H, 10·03. $C_{12}H_{20}O_2$ requires: C, 73·43; H, 10·27%).

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