86. **Tyunosin Ukita, Toru Tsumita, and Nobuyuki Utsugi**: Studies on the Resin Acid in the Fruits of *Juniperus japonica*. The Structure of an Isomeric 7-Isodextropimaric Acid.

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In the course of the studies on the chemical constituents of the fruits of *Juniperus japonica*, two isomeric diterpenic acids, $C_{20}H_{30}O_2$, were isolated and reported in previous papers.[1,2] Further, one of them was identified with 7-isodextropimaric acid (I).[2,3]

This paper deals with the chemical structure of another isomer, $C_{20}H_{30}O_2$, m.p. 162.5~165°, $[\alpha]_D -8.9°$(II), having one carboxyl group and two double bonds.

On catalytic reduction of (II) with platinum in acetic acid, a rapid consumption of one mole of hydrogen was followed by a relatively slow absorption of another mole to give a tetrahydro-acid (III), $C_{20}H_{34}O_2$, m.p. 172.5°, which showed no coloration with tetranitromethane. The product (III) was identified with tetrahydro-7-isodextropimaric acid, $C_{20}H_{34}O_2$, m.p. 172~172.5°, derived from authentic 7-isodextropimaric acid by the same way. Both tetrahydro-acids melted without depression on mixed fusion and gave the same infrared spectrum. Therefore, (II) must also be a pimaric-type acid with the same optical configuration at C_7 as 7-isodextropimaric acid.

As the ultraviolet spectrum of (II) showed no absorption maximum between the wave range of 215 mμ and 300 mμ, which is specific for conjugated double bonds, the two double bonds in (II) must be located apart from each other.

When the original acid (II) was hydrogenated in ethanol solution with 10% palladium carbon catalyst, after a rapid absorption of one mole of hydrogen, a dihydro-acid (IV),[2] $C_{20}H_{32}O_2$, m.p. 178~181°, was obtained. However, (IV) showed a marked depression on mixed fusion with dihydro-7-isodextropimaric acid, m.p. 173~175°, obtainable from (I) in the same way.

Further, (II) was ozonized in chloroform solution to give formaldehyde in a yield of 38.7% of amount calculated as formaldimedone.[4] Thus, one of the two double bonds of (II) is located in the side chain as a vinyl group. The infrared spectrum of (II) also gave the distinct characteristic absorptions for a vinyl group, at 7.11, 10.00, and 10.98 μ (1407, 1000, and 912 cm^{-1}).

However, as the corresponding absorptions were found to have disappeared in the dihydro compound (IV), it is not inconsistent that on mild hydrogenation of (II), the vinyl group was selectively reduced to give the dihydro-acid (IV).

Dihydro-methyl ester (V), derived from (IV) by methylation with diazomethane, was oxidized with selenium dioxide in ethanol. The product was an oily compound (VI), which was further oxidized with sodium dichromate in acetic acid solution to a ketoester, $C_{21}H_{32}O_3$, $b.p_{0.01}$ 165~171°, (VII), (2,4-dinitrophenylhydrazone, m.p. 191~193°). The absorption maximum at 245 mμ ($\log \epsilon$ 3.5) in the ultraviolet spectrum of the ketoester indicates that (VII) has a carbonyl group conjugated with the double

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1) T. Ukita, R. Matsuda: J. Pharm. Soc. Japan, **71**, 1050 (1951).
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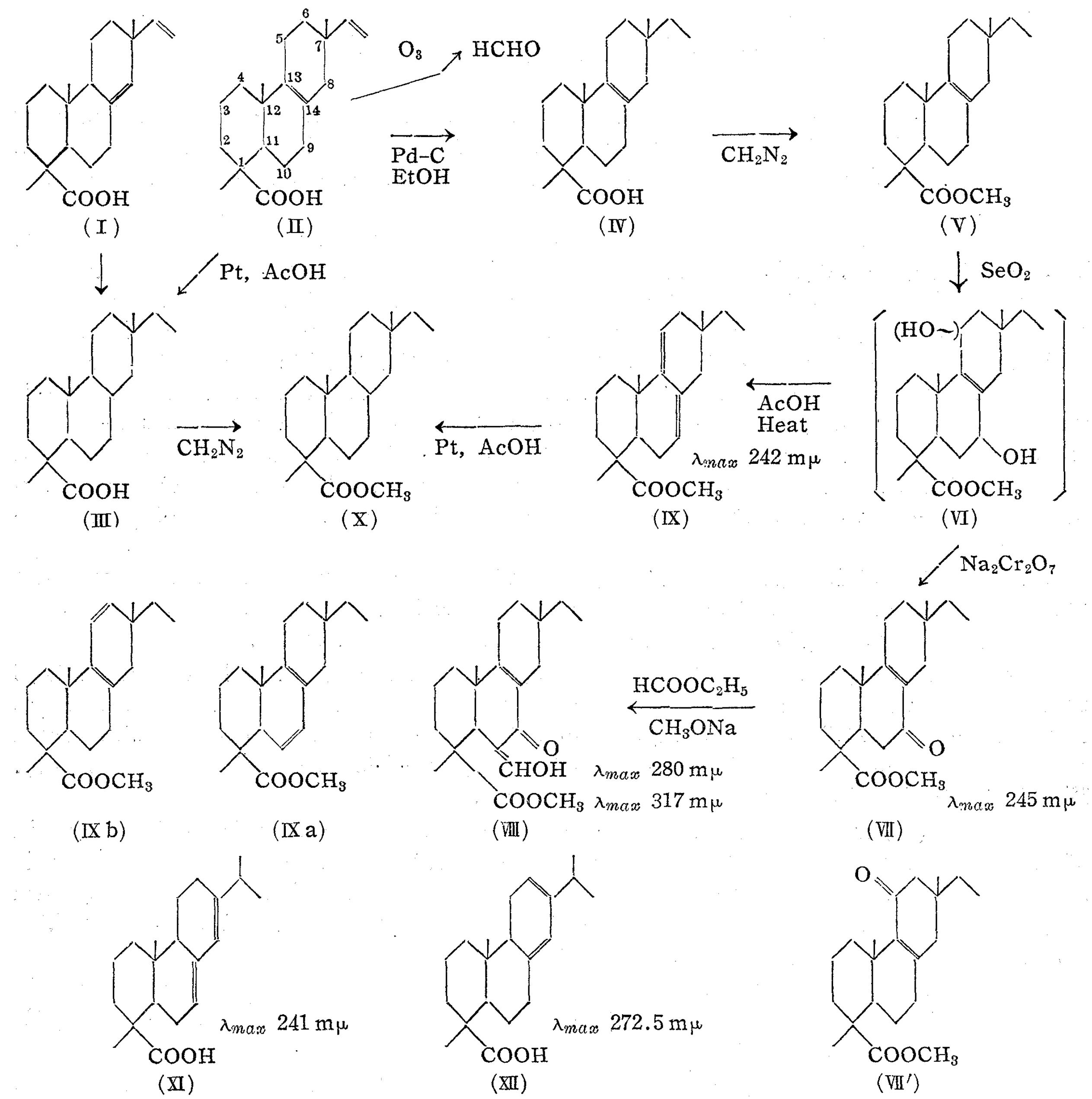
bond and that on primal oxidation of dihydro-ester (V) with selenium dioxide a hydroxyl group was introduced at the neighboring methylene group of the double bond.



(VII) was further treated with ethyl formate and sodium methoxide in anhydrous benzene and the product was isolated as faint yellow crystals (VIII), $C_{22}H_{32}O_4$, m.p. 132~133°, which showed an acidic reaction and gave positive aldehyde reactions with both Schiff's and Tollens' reagents and by ferric chloride test. The ethanol solution of (VIII) showed two absorption bands at 280 mμ (log ε 3.7) and 317 mμ (log ε 3.9) in contrast with the alkaline solution which showed three absorptions at 273 mμ (log ε 3.7), 325 mμ (log ε 3.8) and 397 mμ (log ε 3.4) in ultraviolet range.

Thus, (VIII) is a hydroxymethylene compound. The introduction of a hydroxymethylene group by above reaction on keto-ester (VII) indicates that the latter has a methylene group activated by the carbonyl group. Further, as the carbonyl group of (VII) was derived from a methylene group at the α-position of the double bond in (V), the partial structures $-C=C-CH_2-$ (A), $-C=C-CO-CH_2-$ (B), and $-C=C-CO-\overset{|}{C}=CHOH$ (C) must be involved in the compounds (V), (VII), and (VIII), respectively. The partial structure (A) for dihydro-methyl ester (V), a dextropimaric acid-type compound, is only admissible when the double bond is located at the position of C_{13}—C_{14}.

Therefore, dihydro-methyl ester must be represented by the structure (V), and further the original acid which has one more double bond in the vinyl side chain must reasonably have the structure shown by (II).

In the structure of (V), there are two methylene groups at the α-position of the double bond, C_5 and C_9, both of which are susceptible to the oxidation to give the keto-ester (VII) and (VII′) and fulfil the above partial structure (A) for the dihydro-methyl ester (V). However, from the stereochemical points of view[5] the possibility of (VII) is greater than that of (VII′).

Dehydration occurred easily for hydroxy-methyl ester (VI) by boiling with acetic acid or heating at 190~200° to give needle crystals (IX), $C_{21}H_{32}O_2$, m. p. 75~77°. (IX) consumed two moles of hydrogen on catalytic reduction with platinum to yield tetrahydro-methyl ester (X), $C_{21}H_{36}O_2$, m. p. 69°, which was identified with methyl ester of (III) by mixed fusion. An intense ultraviolet absorption maximum at 242 mμ (log ϵ 4.0) and positive coloration of doubly unsaturated compound (IX) with *p*-nitrobenzenediazonium chloride[6] strongly indicates that the two double bonds of (IX) are in conjugation.

If the dehydration of (VI) gave a new double bond at the α,β-position for the unsaturated linkage of (V), the structure (IXa) or (IXb) could be given for the diene compound. According to the Woodward's rule,[7] both structures (IXa) and (IXb), which contain homoanur conjugation, should give a calculated wave length 273 mμ for the maximum absorption in ultraviolet spectra. However, in contrast with homoanur conjugation the maximum absorption for heteroanur conjugation is reported to occur at 30 mμ shorter wave length than for the former. Thus maximum absorptions at 241 mμ and 272.5 mμ are observed for abietic acid (XI) and levopimaric acid (XII), respectively.[8,9]

As the diene compound (IX) gave a maximum absorption at 242 mμ, its structure would most reasonably be represented by methyl $\Delta^{5,13:9,14}$-7-isodextropimarate.

The formation of such conjugation by dehydration of the compound (VI) is also supported by a similar reaction for a derivative of neoabietic acid (XIII). Harris and Sanderson[10] obtained a diene acid (XV) by the treatment of compound (XIV) with acetyl chloride or dry hydrochloric acid in benzene solution. (XV) also showed an absorption maximum at 242 mμ which is characteristic for heteroanur conjugation. Further, in a similar instance, Ruzicka and his co-workers[11] reported that the oxidation of lanostenol acetate (XVI) by selenium dioxide, followed by dehydration gave a heteroanur diene compound, which was further proved to be identical with dihydroagnosterol acetate, (XVII), λ_{max} 243 mμ (log ϵ 4.2).

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5) Both structures (VII) and (VII′) were tested for their stereochemical stabilities by the Fischer-Taylor-Hirschfelder model.

6) L. F. Fieser, W. P. Campbell : J. Am. Chem. Soc., **60**, 159 (1938).

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10) G. C. Harris, T. E. Sanderson : J. Am. Chem. Soc., **70**, 339 (1948).

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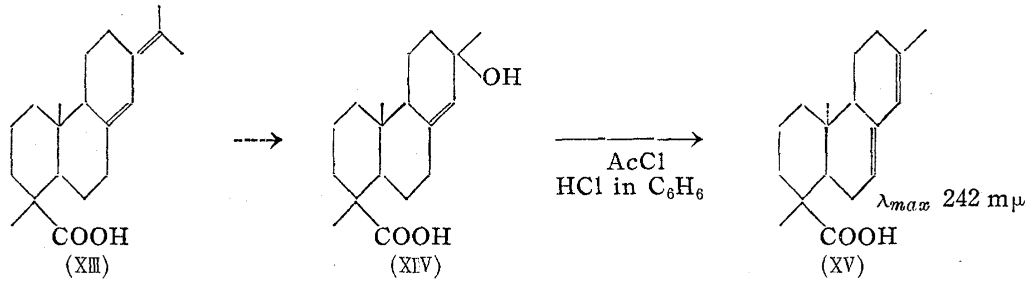
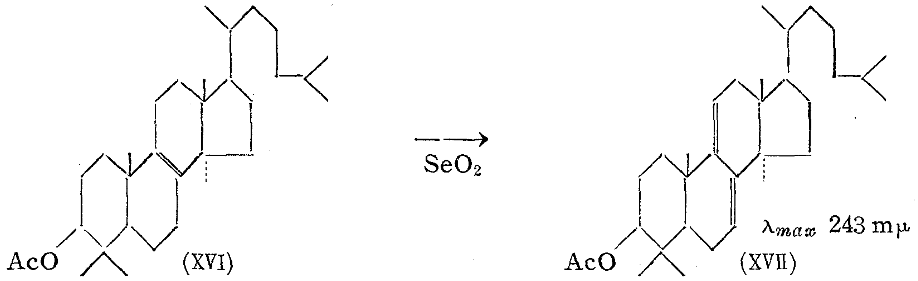
It is of interest that the location of conjugated double bonds is more stable in the case of diterpenic acid as well as triterpenoid when they are arranged heteroanurly in B and C rings than when they are contained in one ring.

In the simultaneous isolation of both $\Delta^{13,14}$-7-isodextropimaric acid (II) and 7-isodextropimaric acid (I) from the extract of Japanese juniper there remains a problem whether one of these two acids were derived from the other by migration of the double bond, as during the isolation both acids were recrystallized from acetic acid or regenerated from their 2-amino-2-methylpropanol salt by mineral acid. However, a change of (I) to (II) or *vice versa* has not been observed when each acid was heated singly in acetic acid solution.



(XIII) (XIV) (XV)



(XVI) (XVII)

The authors wish to thank Mr. H. Kamata, Lecturer of the Applied Chemistry, University of Tokyo, for his help in the measurements of infrared spectra and to Miss. E. Kondo and Mr. B. Kurihara for carrying out the microanalysis.

Experimental

Hydrogenation of (II) to Tetrahydro-acid (III)—0.40 g. of (II) (mp. 162.5~165°) was dissolved in AcOH and the solution was hydrogenated with 0.1 g. of Pt catalyst. One-half of the theoretical volume (63.5 cc. for two moles) of H_2 was absorbed within 20 mins. and after additional 5 hrs. the total consumption of H_2 reached 85% of the calculated amount. The catalyst was removed by filtration and the product was precipitated from the filtrate by addition of water. The dried product was again hydrogenated in the same way with new catalyst until 90% of theoretical amount of H_2 was absorbed. The reaction mixture was filtered from the catalyst and precipitated with water. After repeated recrystallization from MeOH, tetrahydro-acid was obtained as colorless needles (III), m.p. 171~172.5°, which showed negative tetranitromethane test. Yield, 0.27 g. *Anal.* Calcd. for $C_{20}H_{34}O_2$: C 78.43; H, 11.11. Found: C, 78.24; H, 11.23.

Hydrogenation of (I) to Tetrahydro-7-isodextropimaric Acid (III)—0.27 g. of (I) (mp. 160~161°) was hydrogenated similarly until 44.7 cc. of H_2 was absorbed (Calcd. 43.7 cc. for two moles of H_2). The product was crystallized as above to give colorless needles, m.p. 172~172.5°. Yield, 0.2 g. Tetranitromethane did not color an ethanolic solution of the product. *Anal.* Calcd. for $C_{20}H_{34}O_2$: C, 78.43; H, 11.11. Found: C, 78.69; H, 11.00.

A mixed fusion of both tetrahydro-acids melted at 171~172° without depression.

Partial Hydrogenation of (II) to Dihydro-acid (IV)—3.0 g. of (II) dissolved in EtOH was hydrogenated with 10% Pd-C catalyst. The reaction was stopped after 1 hr. when 239.6 cc. (Calcd. 243 cc. for one mole) of H_2 was absorbed. The catalyst was filtered off and washed with ether. The filtrate was combined with washed ether and the solvents were removed to give an oily product, which was recrystallized from EtOH and dil. AcOH, successively.12) Needle crystals,

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12) Repeated recrystallizations were necessary to obtain the pure dihydro-acid free from traces of non-hydrogenated original acid (II). The low melting point, m.p. 152~155°, of the dihydro-acid reported in the previous paper2) was due to contamination with some original acid.

m.p. 178~181°, (IV) (Yield 1.7 g.). *Anal.* Calcd. for $C_{20}H_{32}O_2$: C, 78.94; H, 10.53. Found : C, 78.86; H, 10.64.

Ozonolysis of (**II**)—The ozonized oxygen was passed through a solution of 0.3 g. of (II) in 40 cc. of $CHCl_3$ under cooling with ice water. After 1.5 hrs. the solution was saturated with ozone. The solvent was removed *in vacuo* and ice water was added to the residue. After standing for 30 mins. at room temperature, the mixture was warmed gently and distilled with steam for 2 hrs. 60 cc. of distillate was collected in 2% dimedone solution (ethanol 1 : water 2) under cooling. The crystals formed were filtered, washed with water and dried *in vacuo*. The product melted at 188.5~190°. (Yield, 0.112 g.). Mixed fusion with authentic formaldimedone, m.p. 189~190°, showed no depression.

Preparation of $\Delta^{13,14}$-9-Oxo-7-isodextropimaric Acid (VII)—5.2 g. of (IV) was dissolved in ether and methylated with CH_2N_2 in a usual manner. Dihydro-methyl ester (V), m.p. 61~62°, was recrystallized from dil. EtOH (Yield, 4.8 g.). A solution of 1.2 g. of (V) in EtOH was added into the solution of 0.4 g. of SeO_2 in EtOH containing a small amount of water. The mixture was set aside at 37° overnight after standing for 4 hrs. at room temperature. The supernatant was decanted from the reaction mixture into a large volume of water and extracted with ether. The ether extract was washed with water, dried over Na_2SO_4, and decolorized twice with charcoal. On evaporation of the ether, a faint yellow oil (VI) was obtained. Without further purification, (VI) was dissolved in 100 cc. of AcOH and oxidized by dropwise addition of 0.25 g. of $Na_2Cr_2O_7$ dissolved in 30 cc. of AcOH. The reaction was carried for 4 hrs. under stirring at room temperature. The excess $Na_2Cr_2O_7$ was decomposed with MeOH and the supernatant liquid was decanted into a large volume of water and extracted with ether. The ether extract was shaken with 5% NaOH solution, 5% HCl, and water, successively, and dried over Na_2SO_4. A resinous oil, obtained on evaporation of solvent, was fractionally distilled to give the main product, which boiled at 160~170° under 0.01 mm. On repeated distillation, the product showed $b.p_{0.01}$ 165~171° and λ_{max} 245 mμ (log ε 3.5). (VII). (Yield, 0.32 g.). *Anal.* Calcd. for $C_{21}H_{32}O_3$: C, 75.90; H, 9.64. Found : C, 76.21; H, 9.70.

2,4-dinitrophenylhydrazone of (VII), m.p. 191~193°. *Anal.* Calcd. for $C_{27}H_{36}O_6N_4$: N, 10.94. Found : N, 11.03.

Preparation of (**VIII**)—0.1 g. of Na was dissolved in anhyd. MeOH and dried at 150° *in vacuo* in N_2 atmosphere. In order to remove traces MeOH, NaOMe was added with anhyd. benzene and the benzene was evaporated exhaustively. After repeating above treatment 5 times, NaOMe was added with 0.2 g. of HCOOEt dissolved in 10 cc. of anhyd. benzene under stirring, a solution of 0.23 g. of (VII) in 20 cc. of anhyd. benzene was added dropwise to the warm mixture, within 3 hrs. An orange colored precipitate formed and the mixture was heated for an additional 2 hrs. After cooling, the reaction mixture was repeatedly extracted with 5% NaOH solution until the alkaline extract became colorless. All the alkaline extracts were combined and acidified with dil. HCl. The precipitate produced was extracted with ether, the ether solution washed with water, and the solvent evaporated after drying. The residue was a brown oil (80 mg.), which was recrystallized from an ether-petroleum ether mixture to faint yellow fine prisms, m.p. 132~133°, (VIII). $FeCl_3$ colored an ethanolic solution of the product dark green. Schiff's test and silver mirror test were also positive. *Anal.* Calcd. for $C_{22}H_{32}O_4$: C, 73.33; H, 8.88. Found : C, 73.69; H, 9.27.

The absorption spectrum of (VIII) showed maxima in mμ at 280 (log ε 3.7) and 317 (log ε 3.9) in EtOH solution and 278 (log ε 3.7), 325 (log ε 3.8) and 397 (log ε 3.4) in ethanolic NaOH solution (1%).

Preparation of (**IX**)—0.1 g. of (VI) dissolved in 3 cc. of AcOH was refluxed for 1 hr. in an oil bath. After cooling, the reaction mixture was diluted with water, neutralized with $NaHCO_3$, and the mixture was extracted with ether. The ether solution was washed with water, evaporated to dryness. The residual oil was distilled *in vacuo* to give a colorless distillate, $b.p_{0.01}$ 155~160°, which was recrystallized from MeOH and AcOH to needles (IX), m.p. 75~77°. *Anal.* Calcd. for $C_{21}H_{32}O_2$: C, 79.75; H, 10.13. Found : C, 79.70; H, 10.18.

The absorption spectrum of (IX) showed maximum in mμ at 242 (log ε 4.0) in EtOH solution. An acetic acid solution of (IX) colored deep orange within 30 mins. by addition of *p*-nitrobenzenediazonium chloride.

Hydrogenation of (**IX**)—9.14 mg. of (IX) dissolved in AcOH was hydrogenated with Pt catalyst using the Warburg apparatus at 37°. Calculated amounts of H_2 (1.55 cc. at 37° for two moles) were absorbed within 3 hrs. After removal of the catalyst, the filtrate was poured into water. The acidic solution was neutralized with $NaHCO_3$ and shaken with ether and the ether layer was washed with water. On removal of the solvent, a residual colorless oil solidified into a crystalline mass which was recrystallized from dil. MeOH to needles (X), m.p. 69°. (X) showed no coloration with tetranitromethane.

The methylation of (III) with CH_2N_2 gave methyl tetrahydro-7-isodextropimarate, m.p. 69°

(X). *Anal.* Calcd. for $C_{21}H_{36}O_2$: C, 78.75; H, 11.25. Found : C, 79.00; H, 11.02.

A mixture of both methyl esters melted without depression.

Summary

By successive oxidation with selenium dioxide and sodium dichromate followed by introdution of a hydroxymethylene group, a partial structure $-C=C-CH_2-CH_2-$ was given for a diterpenic acid $C_{20}H_{30}O_2$ (II) which was isolated from the fruits of Japanese juniper. Further, as the acid (II) gave tetrahydro-7-isodextropimaric acid after consumption of two moles of hydrogen, the structure of (II) was concluded to be represented by $\Delta^{13,14}$-7-isodextropimaric acid.

The structure of a doubly unsaturated compound obtainable from a hydroxymethyl ester (VI), an oxidation product of (V), was proposed to be $\Delta^{5,13:9,14}$-7-isodextropimaric acid.

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87. **Masaichiro Masui and Takami Teshima**: High Frequency Titration. XIV.* Estimation of the Distance between the Two Carboxyl Groups of a Dicarboxylic Acid by High Frequency Titration.

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The theoretical relation between the dissociation constants and the distance, r, of the two carboxyl groups in a dicarboxylic acid was first studied by Bjerrum.[1] His expression was based upon a rather simple calculation but the data obtained by his method using the dissociation constant of a dicarboxylic acid were unsuitable, especially where r was small. The modified method[2] or the same calculation with the different dissociation constants[3] were reported later, but the values of r were still somewhat doubtful, because some assumptions included in the method were not suited to the actual conditions. The most reliable theoretical method of calculation for r was presented by Kirkwood, Westheimer, and Shookhoff,[4~7] though it was, in its true object, an argument for the electrostatic influence of substituents on the dissociation constants of organic acids. Therefore, their method was rather complicated by the use of the factor D_E (effective dielectric constant), K_1 and K_2 (the first and second dissociation constants), and cos ξ (the angle between the dipole and the line which combines the center and the ionized proton).

In our study of the high frequency titration of organic acids in nonaqueous medium,[8] one of the present writers (M) found that some dicarboxylic acids showed an N-shaped titration curve in a certain medium, and a simple estimation method of the distance r between the two carboxyl groups in dicarboxylic acids was deduced

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* This work is a part of series entitled "High Frequency Titration" by M. Ishidate. Part XIII. : J. Pharm. Soc. Japan, **75**, 1519(1955).

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