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The Methylation of Stipitatic Acid with Diazomethane

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Methylation of stipitatic acid with diazomethane gave three isomeric dimethyl ethers; 2,4-, 2,6- and 3,4-dimethoxytropone derivatives. Their structures were elucidated by the utilization of nuclear magnetic resonance spectra and some chemical reactions.

In general, the tropolone is interpreted as a highly mobile tautomeric system due to the shift of hydrogen between carbonyl at C-1 position and hydroxyl at C-2 position.[2]

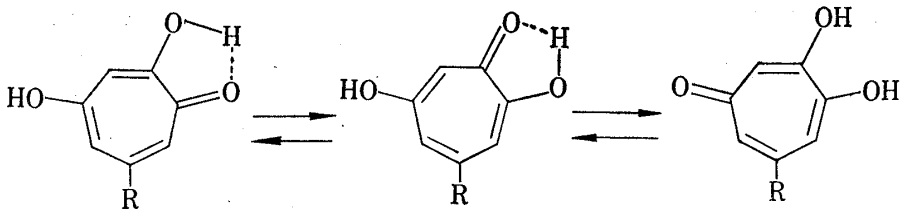


Chart 1

In case of the tropolone with hydroxyl group as a substituent, the situation becomes more complicated, because of an extra tautomerism. For example, in case of 4-hydroxytropolone, the tautomerism as shown in Chart 1 may be expected. If it is the case, on the methylation with diazomethane three kinds of the dimethyl ether derivatives may be formed. Recently it was reported by us and Canadian workers that anhydrosepedonin, a metabolite of *Sepedonium chrysospermum*, and its derivatives which are the 4-hydroxytropolone derivatives, gave three isomeric dimethyl ethers.[3,4] On the other hand, in case of stipitatic acid (I) (Chart 1, R=COOH), a metabolite of *Penicillium stipitatum*, it was reported that I gave only two isomeric dimethyl ethers by the action of diazomethane,[5] which were believed to be 2,6-dimethoxy- and 2,4-dimethoxytropone derivatives.[2] However, in this case also, the formation of 3,4-dimethoxytropone derivative was expected similarly to the case of anhydrosepedonin. The present paper describes the isolation of three kinds of the dimethoxytropone derivatives and their structural elucidation.

Stipitatic acid (I) was isolated from the culture of *Penicillium stipitatum* by Raistrick, *et al.*,[6] and its structure was supposed by Dewar[7] in the early period of tropolone chemistry and confirmed by the synthesis.[8] The biochemical studies were also carried out.[9] The

1) Location: *Katahira-2-chome, Sendai.*
2) T. Nozoe, K. Takase, H. Matsumura, T. Asao, K. Kikuchi and S. Ito, "Dai Yuki Kagaku, (Comprehensive Organic Chemistry)," Vol. 13, Asakura Shoten, Tokyo, 1960.
3) S. Takenaka and S. Seto, *Sci. Rep. RITU*, A-Vol. **21**, 106 (1969).
4) J.L.C. Wright, A.G. McInnes, D.G. Smith and L.C. Vining, *Can. J. Chem.*, **48**, 2703 (1970).
5) J.H. Birkinshaw and H. Raistrick, *Biochem. J.*, **36**, 242 (1942).
6) J.H. Birkinshaw and H. Raistrick, *Biochem. J.*, **26**, 441 (1932); G. Barger and O. Dorrer, *ibid.*, **28**, 11 (1934).
7) M.J.S. Dewar, *Nature*, **155**, 50 (1945).
8) J.R. Bartels-Keith, A.W. Johnson and W.I. Taylor, *Chem. Ind.* (London), **1951**, 337; R.B. Johns, A.W. Johnson and J. Murray, *J. Chem. Soc.*, **1954**, 198.
9) R. Bentley, *J. Biol. Chem.*, **238**, 1889, 1895 (1963).

sample (I) used in this study was obtained as pale yellow plates, mp 302—304°, by the method of Bentley, *et al.*[10] In its infrared (IR) spectrum the broad band between 3000—2500 cm^{-1} shows the presence of strong hydrogen bond. The nuclear magnetic resonance (NMR) spectrum of I in 6*d*-DMSO at 30° shows the signals at 6.89 (1H, d, J=2.9 Hz), 7.44 (1H, d, J=1.5 Hz) and 7.52 ppm (1H, dd, J=2.9 and 1.5 Hz). These three signals are assigned to the ring-protons.

The compound (I) was methylated with diazomethane and the products were separated through an alumina column. Three isomeric dimethyl ethers were referred to as IA, IB and IC, according to the order of the elution. The methylation of I in methanol at 0° gave IA and IC as the major products and IB as minor one. In this case, the photometric measurement of the crude material showed a ratio of these components to be 1: 1: 2.6, suggesting that during the separation a part of IB was lost. The methylation at −60° gave IC and a very small amount of IA. The melting points of IA, IB and IC were 133—134°, 176—177° and 184—186°, respectively. The consideration on these melting points and the formation ratio suggests that the two isomeric dimethyl ethers obtained by Raistrick, *et al.*[5] may be IA and IC.

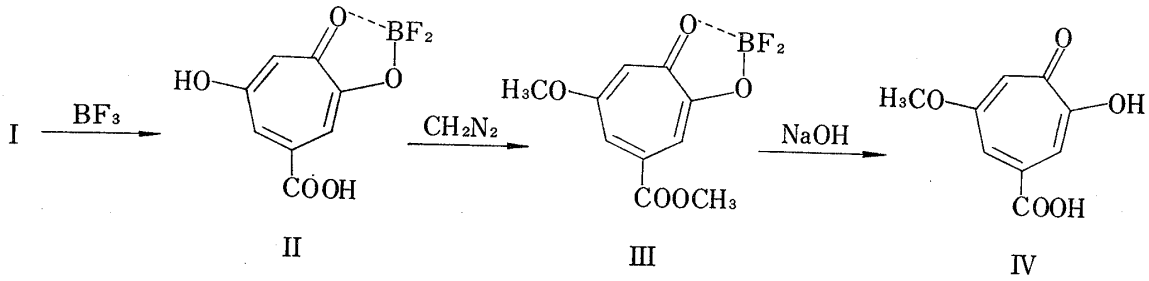


Chart 2. Preparation of 4-Methoxytropolone Derivative (IV)

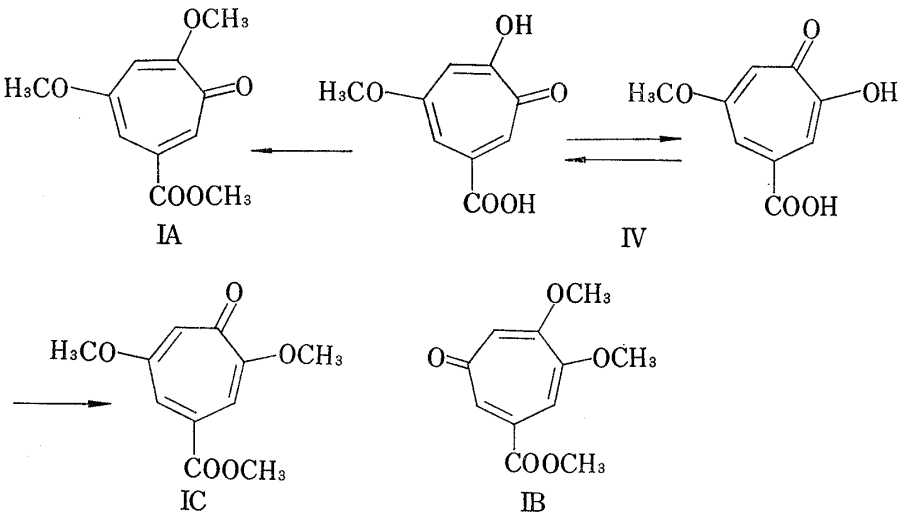


Chart 3

Then, 4-methoxytropolone derivative (IV) was prepared through a relevant route (*via* the difluoroboron derivative[3,11,12]), as shown in Chart 2. The NMR spectrum of IV in 6*d*-DMSO showed the signals at 3.90 (3H, s), 6.93 (1H, d, J=2.7 Hz) and around 7.49 ppm (2H, broad). The methylation of IV gave IA and IC, but not IB, suggesting that the structure

10) R. Bentley and C.P. Tiesen, *J. Biol. Chem.*, **238**, 1880 (1963).
11) J.W. Cook, R.A. Raphael and A.I. Scott, *J. Chem. Soc.*, **1952**, 4416.
12) S. Seto, S. Matsumura and K. Ro, *Chem. Pharm. Bull.* (Tokyo), **10**, 901 (1962).

of IB was 3,4-dimethoxy-6-methoxycarbonyltropone. However, it cannot yet be decided which of IA and IC would correspond to 2,4-dimethoxytropone derivative or 2,6-dimethoxytropone derivative. In order to clarify the correlation, IA and IC were derived to the corresponding chlorotropone derivatives (VI and VIII), respectively, as shown in Chart 4. The reaction of IA with hydrazine at room temperature gave V and thereby methoxycarbonyl group did not react with hydrazine under such a mild condition. Then the decomposition of the hydrochloride of V in a boiling aqueous solution of cupric sulfate gave VI.[13] The isomeric chloro compound (VIII) was obtained in the similar manner, *via* the hydrazino compound (VII) starting from IC. The ultraviolet (UV) spectra of VI and VIII showed very similar patterns to those of 4-methoxytropone[14] and 2-chloro-3-methoxytropone,[13] respectively. From these results IA and IC were assumed to be 2,4-dimethoxy-6-methoxycarbonyltropone and 2,6-dimethoxy-4-methoxycarbonyltropone, respectively.

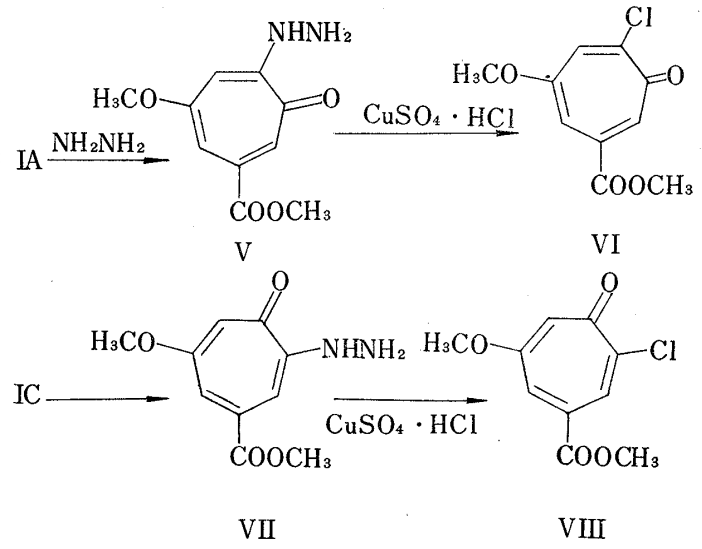


Chart 4. Preparation of the Chlorotropone Derivatives (VI and VIII)

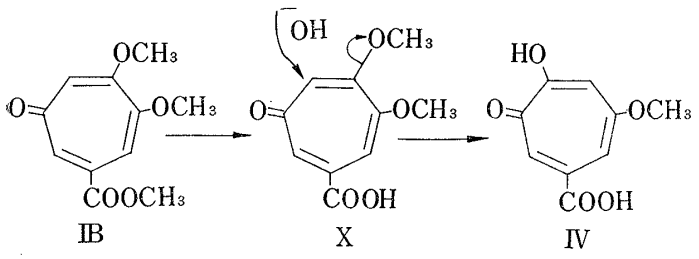


Chart 5

The reaction of IB with hydrazine under the same condition as mentioned above gave a monohydrazino derivative (IX). The NMR spectrum of IX showed the signals at 3.89 and 3.90 ppm (3H, s, respectively) due to two methyl groups and 6.67 (1H, d), 6.74 (1H, d) and 7.32 ppm (1H, dd) due to three ring-protons, and the UV spectrum showed the maxima at 283 (log ε=4.56), 341 (3.81) and 425 mμ (3.56). The pattern of the ultraviolet (UV) spectrum was very similar to that of 3-aminotropone.[15] From these facts, the structure of IX is assumed to be 3-hydrazino-4-methoxy-6-methoxycarbonyltropone.

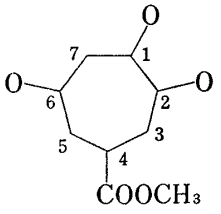
The hydrolysis of IA and IC with 6N sodium hydroxide gave IV, while IB gave also a carboxylic acid (IV'). The melting point of IV' differed a little from that of IV, but its NMR spectrum agreeded with that of IV. The compound IV' showed the coloration with ferric chloride, suggesting that IV' was a tropolone derivative. From these results, IV' was assumed to be identical with IV, and the difference may be due to the polymorphism. The formation of IV from IB must be attributed to an abnormal substitution reaction[2] which occurs often in the tropone derivative. The mild treatment of IB with 1N sodium hydroxide gave an acid (X), whose analytical values agreeded with $C_{10}H_{10}O_5$, and its UV spectrum showed the maxima at 251 (log ε=4.42) and 337 mμ (3.79). The NMR spectrum of X in 6*d*-DMSO exhibited the signals at 3.90 and 3.93 ppm (3H, s, respectively) and 6.61 (1H, d, J=3.0 Hz), 6.89

13) S. Seto, *Sci. Repts. Tohoku Univ.*, First Series, **37**, 276 (1953).
14) O.L. Chapman, *J. Am. Chem. Soc.*, **80**, 633 (1958).
15) S. Seto, T. Hiratsuka and H. Toda, *Yakugaku Zasshi*, **89**, 1673 (1969).

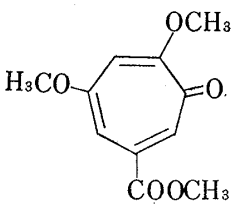
(1H, d, J=1.6 Hz) and 7.34 ppm (1H, dd, J=3.0 and 1.6 Hz). These facts suggested that the structure of X was 3,4-dimethoxy-6-carboxytropone.

The NMR Spectra of IA, IB and IC

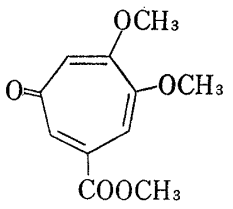
The structures of these compounds elucidated chemically as described above, are also supported by the consideration of the NMR spectra. In order to avoid the confusion of the skeletal numbering, the provisional numbering for the explanation of the NMR spectra is used regardless of the nomenclature, as shown in Chart 6.



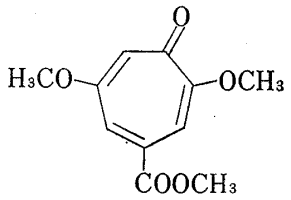
numdering for the NMR spectra



IA



IB



IC

Chart 6

TABLE I. The Chemical Shifts (δ, ppm) and Coupling Constants (Hz) of IA, IB and IC in $CDCl_3$ with Tetramethylsilane as an Internal Standard

	1 -OCH_3	2 -OCH_3	3 -H	4 -$COOCH_3$	5 -H	6 -OCH_3	7 -H
IA	3.95		7.80 J=1.4	4.01 4.00	7.00 J=1.4 J=2.5	4.00 4.01	6.71 J=2.5
IB	3.99	3.95	6.69 J=2.3	3.99	7.65 J=1.3 J=2.3		7.05 J=1.3
IC		3.88	6.82 J=2.8	4.03	7.67 J=2.8 J=1.3	4.02	7.31 J=1.3

The NMR spectra in $CDCl_3$ are shown in Figures 1, 2 and 3. The assignment of each signal was carried out in the same manner as the case of the anhydrosepedonin derivatives,[3] the chemical shifts, the *meta*-coupling and the long-range coupling of the methoxyl group[16] being taken into account. The signals which appear around 4 ppm are assigned to the protons of methoxyl and methoxycarbonyl groups. The signals due to three ring-protons in each isomer appear as a double doublet and two doublets, one of which has a very small coupling constant and the other a little larger coupling constant. The double doublet should be attributed to C-5 proton, because it is doubly *meta*-coupled. Many of the signals due to ring-protons are a little broadened by the long-range coupling with the methoxyl protons. Careful decoupling technique by irradiation at methoxyl region makes possible to assign the signals due to C-3 and C-7 protons. For example, in case of IA (see Fig. 1), the irradiation at frequencies of methoxyl signal at 3.95 ppm sharpens the signal at 6.71 ppm, and the irradiation at 4.01 ppm does both signals at 6.71 and 7.00 ppm. The signal at 7.80 ppm is not affected by these irradiations. These results suggest that the signals at 3.95, 4.01, 6.71, 7.00 and 7.80 ppm are due to the protons of C-1 methoxyl, C-6 methoxyl, C-7, C-5 and C-3, respectively. The signal at 4.00—4.01 ppm is superimposed with the signal of methoxycarbonyl protons.

16) S. Seto, K. Ogura, H. Toda, Y. Ikegami and T. Ikenoue, *Bull. Chem. Soc. Japan*, **41**, 2696 (1968).

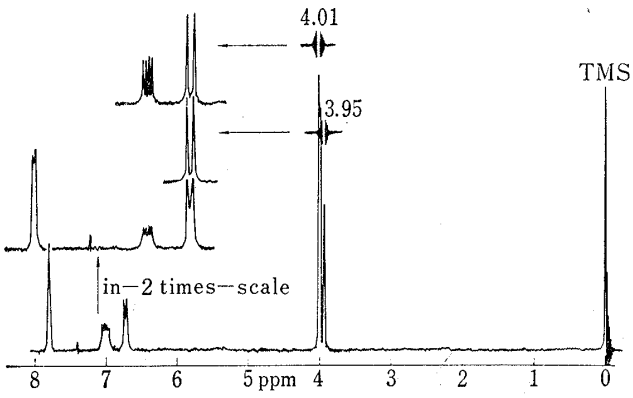


Fig. 1. The NMR Spectrum of IA in $CDCl_3$

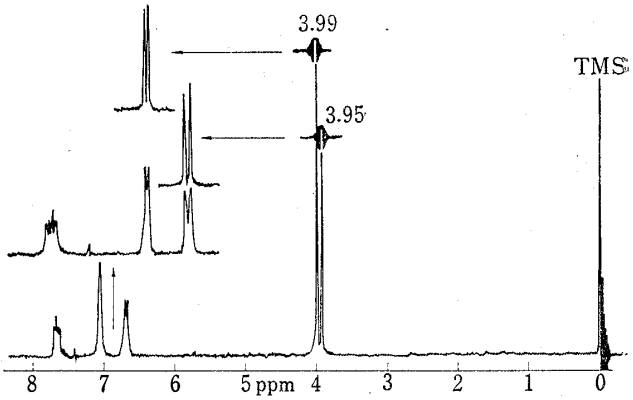


Fig. 2. The NMR Spectrum of IB in $CDCl_3$

In case of IC, the assignment is possible in the similar manner (see Fig. 3). However, in case of IB, the irradiation at the methoxyl proton reveals the structure, but not the correlation between the signals (at 6.69 and 7.05 ppm) and the ring-protons other than that at C-5. As shown in Fig. 2, the irradiation at 3.95 ppm sharpens the signal at 6.69 ppm, the irradiation at 3.99 ppm does the signal at 7.05 ppm, but both irradiations does not affect the signal at 7.65 ppm which is assigned to C-5 proton from the splitting pattern. These phenomena would occur only in case of IB. In order to correlate each signal at 6.69 and 7.05 ppm to the respective position, inspection of the values of *meta*-coupling constant might be useful. In IA and IC, the *meta*-coupling constant of the ring-proton neighboring to the carbonyl group of the tropone skeleton ($J_{3,5}$ in IA and $J_{5,7}$ in IC) is smaller than that of the other ring-proton ($J_{5,7}$ in IA and $J_{3,5}$ in IC). If this correlation is applicable to the case of IB, the signal at 7.05 ppm which exhibits a smaller coupling constant may be assigned to the proton at C-7.

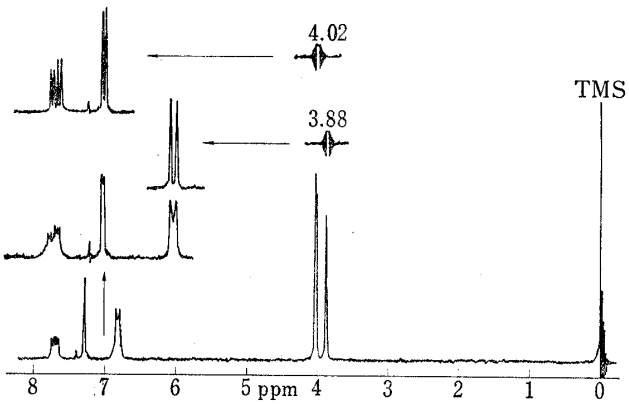


Fig. 3. The NMR Spectrum of IC in $CDCl_3$

These results are summarized in Table I. From Table I, the following is noticed: the signal of the ring-proton at the double bond which is conjugated with the methoxycarbonyl group (C-3 proton in IA, C-5 protons in both IB and IC) appears at the lowest field. If these features are available for the NMR spectrum of stipitatic acid (I) itself in the DMSO solution, it may be assumed that the IC type tautomer predominates among the three tautomers.

Experimental[17]

Stipitatic Acid (I)——I was isolated from the cultures of *Penicillium stipitatum* NRRL 1006 and NHL 6092 according to the method of Bentley.[10]

17) All melting points are uncorrected. The measurements of UV and IR spectra were made with a Cary recording spectrometer model 14 and with a Hitachi EPI-G2 spectrophotometer, respectively. NMR spectra were measured on a Hitachi H-60 spectrometer, a Varian HA-100 spectrometer and a JOEL C-60-HL spectrometer with tetramethylsilane as an internal standard. The abbreviations used are as follows: s, singlet; d, doublet; dd, double doublet.

Methylation of I with Diazomethane——To a suspension of I (1.0 g) in a small amount of methanol, an ethereal solution of diazomethane was added until a portion of the solution was no longer colored with ferric chloride, and the resulting mixture was immediately evaporated to dryness under a reduced pressure. The slightly greenish residue was applied to a top of an alumina column which was beforehand deactivated with ethyl acetate, and chromatographed by elution with ether. The first fraction gave IA as pale yellow microcrystals after recrystallization from a mixture of ether and ethyl acetate. The second fraction which was obtained by elution with 5% ethyl acetate–ether, gave IB as pale yellow crystals after recrystallization from ethyl acetate. The third fraction which was obtained by elution with 10% ethyl acetate–ether, gave IC as colorless needles after recrystallization from ethyl acetate or methanol. IA; mp 133—134°, yield 253 mg. *Anal.* Calcd. for $C_{11}H_{12}O_5$: C, 59.10; H, 5.23. Found: C, 58.92; H, 5.40. UV λ_{max}^{MeOH} mμ (log ε): 242 (4.41), 327 (3.73), 372 (3.98). IR ν_{max}^{KBr} cm^{-1}: 1618, 1600, 1578, 1510. Mass Spectrum *m/e*: 224 (M^+). IB; mp 176—177°, yield 68 mg. *Anal.* Calcd. for $C_{11}H_{12}O_5$: C, 59.10; H, 5.23. Found: C, 59.16; H, 5.53. UV λ_{max}^{MeOH} mμ (log ε): 248 (4.37), 340 (3.82). IR ν_{max}^{KBr} cm^{-1}: 1632, 1586, 1560. Mass Spectrum *m/e*: 224 (M^+). IC; mp 184—186°, yield 318 mg. *Anal.* Calcd. for $C_{11}H_{12}O_5$: C, 59.10; H, 5.23. Found: C, 58.84; H, 5.08. UV λ_{max}^{MeOH} mμ (log ε): 257 (4.56), 349 (3.73). IR ν_{max}^{KBr} cm^{-1}: 1632, 1605, 1595, 1542. Mass Spectrum *m/e*: 224 (M^+).

Difluoroboron Compound (II)——To a suspension of I (600 mg) in chloroform (30 ml) was added 1 ml of borontrifluoride etherate. The resulting mixture was allowed to stand overnight at 4°, and evaporated to dryness under a reduced pressure. The residue was triturated with a small amount of water, collected by filtration and washed with water. Then II was obtained as a colorless powder, mp 217—220° (decomp.), yield 587 mg. UV λ_{max}^{MeOH} mμ (log ε): 253 (4.40), 362 (3.65).

Methylation of II——To a suspension of II (500 mg) in 0.3 ml of methanol was added an ethereal solution of diazomethane until evolution of nitrogen had ceased. After standing overnight, the reaction mixture was evaporated under a reduced pressure and the residue was recrystallized from methanol to give III, mp 120—122°, yield 357 mg. UV λ_{max}^{MeOH} mμ (log ε): 256 (4.45), 363 (3.68).

Hydrolysis of III——A solution of III (300 mg) in a mixture of methanol (5 ml) and 6N NaOH (2 ml) was heated on a water bath for 2 hr. To the reaction mixture, water (10 ml) was added and a small amount of insoluble material which precipitated, was removed by filtration. The filtrate was acidified with 6N HCl, and allowed to stand overnight. The precipitate was collected by filtration to give IV, mp 240—245°, yield 220 mg. Its physical data were identical with those of IV obtained by hydrolysis of IA and IC.

Methylation of IV——To a suspension of IV (200 mg) in methanol (10 ml) was added an ethereal solution of diazomethane until a portion of the reaction mixture did not show any coloration with ferric chloride. The evaporation of the reaction mixture gave a crystalline residue. The chromatographic purification of the residue in the similar manner to the case of the methylation of I, gave 10 mg of IA and 95 mg of IC.

2-Hydrazino-4-methoxy-6-methoxycarbonyltropone (V)——A mixture of IA (252 mg), methanol (7 ml) and hydrazine hydrate (70 mg) was stirred overnight at room temperature. The precipitate separated out, was collected and recrystallization from methanol gave orange needles (117 mg), mp 184—185°. *Anal.* Calcd. for $C_{10}H_{12}O_4N_2$: C, 53.57; H, 5.39; N, 12.50. Found: C, 54.01; H, 4.63; N, 12.33. UV λ_{max}^{MeOH} mμ (log ε): 285 (4.35), 347 (3.77), 425 (3.83).

2-Chloro-4-methoxy-6-methoxycarbonyltropone (VI)——To a boiled aqueous solution of cupric sulfate (1 g) in water (2 ml), V (80 mg) in conc. HCl (1 ml) was added at once. After the evolution of nitrogen had ceased, the cooled solution was extracted with chloroform, and the organic layer was washed with a small amount of water and dried over sodium sulfate. Then the solution was treated with active charcoal and evaporated to dryness. The residue was sublimed *in vacuo* (0.001 mmHg), to give VI (25 mg), mp 123—124°. *Anal.* Calcd. for $C_{10}H_9O_4Cl$: C, 52.54; H, 3.97. Found: C, 52.38; H, 3.86. UV λ_{max}^{MeOH} mμ (log ε): 244 (4.31), 357 (3.94). NMR in $CDCl_3$ ppm: 3.50 (3H, s), 3.87 (3H, s), 7.07 (1H, dd), 7.76 (1H, d), 7.81 (1H, d).

2-Hydrazino-4-methoxycarbonyl-6-methoxytropone (VII)——A mixture of IC (110 mg), methanol (11 ml) and hydrazine hydrate (70 mg) was stirred overnight at room temperature. The resulting solution was evaporated under a reduced pressure and the precipitate was collected. Recrystallization from methanol gave VII (80 mg), as yellowish orange crystals, mp 183—184°. *Anal.* Calcd. for $C_{10}H_{12}O_4N_2$: C, 53.57; H, 5.39; N, 12.50. Found: C, 52.43; H, 5.74; N, 12.76. UV λ_{max}^{MeOH} mμ (log ε): 265 (4.34), 346 (3.75), 410 (3.28).

2-Chloro-4-methoxycarbonyl-6-methoxytropone (VIII)——VIII (30 mg) was obtained from VII (150 mg) in a similar manner in case of VI, as crystals, mp 131—132°. *Anal.* Calcd. for $C_{10}H_9O_4Cl$: C, 52.54; H, 3.97. Found: C, 52.34; H, 4.06. UV λ_{max}^{MeOH} mμ (log ε): 257 (4.50), 325 (3.51). NMR in $CDCl_3$ ppm: 3.87 (3H, s), 4.00 (3H, s), 6.75 (1H, d), 7.37 (1H, dd), 8.34 (1H, d).

3-Hydrazino-4-methoxy-6-methoxycarbonyltropone (IX)——A mixture of IB (110 mg), methanol (10 ml) and hydrazine hydrate (30 mg) was stirred overnight at room temperature. Then the reaction mixture was evaporated under a reduced pressure at room temperature. The precipitate thereby obtained was collected and recrystallization from methanol afforded IX (30 mg), mp 156—159°. *Anal.* Calcd. for $C_{10}H_{12}O_4N_2$: N, 12.50. Found: N, 12.13.

Hydrolysis of IA——A solution of IA (150 mg) in 2 ml of 6N NaOH was heated on a water-bath for 2 hr and the resulting solution was acidified with 6N HCl. The precipitate thereby formed, was collected and recrystallized from methanol to give IV (122 mg), mp 243—245° (decomp.). *Anal.* Calcd. for $C_9H_8O_5$: C, 55.10; H, 4.11. Found: C, 54.70; H, 3.97. UV λ_{max}^{MeOH} mμ (log ε): 253 (4.49), 320 (3.59 inf.), 356 (3.64).

Hydrolysis of IC——The same treatment of IC (52 mg) with 6N NaOH as above gave IV (46 mg).

Hydrolysis of IB——The same treatment of IB (44 mg) with 6N NaOH as above gave IV (35 mg).

Mild Hydrolysis of IB——A solution of IB (40 mg) in 1N NaOH (1 ml) was stirred for 2 hr at room temperature. The resulting solution was acidified with 6N HCl and the precipitate was collected. Recrystallization from methanol gave 3,4-dimethoxy-6-carboxytropone (X), 30 mg, mp 236—237°. *Anal.* Calcd. for $C_{10}H_{10}O_5 \cdot H_2O$: C, 52.63; H, 5.30. Found: C, 52.37; H, 5.52. UV λ_{max}^{MeOH} mμ (log ε): 251 (4.42), 337 (3.79).

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