[Chem. Pharm. Bull.
29(8)2182—2187(1981)]

The Structures of Yellow Pigments from the Rhizomes of *Cimicifuga dahurica* MAXIM.[1]

KIMIYE BABA,* MITSUGI KOZAWA, KIYOSHI HATA,
TOSHIMASA ISHIDA and MASATOSHI INOUE

Osaka College of Pharmacy, Kawai-2-10-65, Matsubara, Osaka, 580, Japan

(Received December 11, 1980)

Two yellow pigments, I and II were isolated from the rhizomes of *Cimicifuga dahurica* MAXIM. (Ranunculaceae) and were elucidated as geometric isomers, (*E*)-3-(3′-methyl-2′-butenylidene)-2-indolinone and (*Z*)-3-(3′-methyl-2′-butenylidene)-2-indolinone, by chemical and spectral studies and by X-ray diffraction analysis, respectively. On standing, an ethanolic solution of II gave a colorless substance, which was regarded as a product of dimerization.

Keywords——yellow pigment; *Cimicifuga dahurica*; isatin; (*E*)-3-(3′-methyl-2′-butenylidene)-2-indolinone; (*Z*)-3-(3′-methyl-2′-butenylidene)-2-indolinone; 3-(3′-methylbutyl)-2-indolinone; X-ray analysis of (*E*)-3-(3′-methyl-2′-butenylidene)-2-indolinone

The Chinese drug "Bei Sheng Ma" (Japanese name: Kitashyoma), consisting of the rhizomes of *Cimicifuga dahurica* MAXIM. (Ranunculaceae), has been shown to contain phenolic acids, such as isoferulic acid,[2] triterpenes,[3] and chromones, *e.g.* visamminol, visnagin and so on.[4] The authors have recently detected several yellow pigments in the rhizomes by means of thin-layer chromatography and have tried to isolate these substances, obtaining two products, I and II, as yellow needles, together with isoferulic acid and cimifugin. This paper deals with the structure elucidation of these products.

The ethyl acetate extract of the crude drug, obtained from the Tian-jin market (China), was chromatographed over silica gel using hexane–ethyl acetate (3: 1) as the solvent. The latter half of the eluate upon rechromatography over alumina with hexane–ethyl acetate (2: 1) gave I and II.

Compound I, mp 200—203 °C, was assigned the molecular formula $C_{13}H_{13}NO$ on the basis of analytical and mass spectral data. I was insoluble in aqueous hydrochloric acid and gave no reaction with Dragendorff's test solution. The infrared (IR) spectrum of I was suggestive of the presence of NH and lactam carbonyl groups as well as an aromatic ring and the ultraviolet (UV) spectrum of I showed absorption maxima at 269 nm, 330 nm and 376 nm. The proton magnetic resonance (PMR) spectrum of I showed signals which were assigned to $=C(CH_3)_2$ (δ 2.07, s), =CH–CH= (δ 6.77, 7.59, d, J=12 Hz), NH (δ 8.33, br. s) and four aromatic protons (δ 6.9—7.6, m).

Ozonolysis of I led to the formation of a red crystalline product (III), mp 198—199 °C, which was identified as isatin by mixed melting point determination with an authentic sample and comparison of spectral data. Furthermore, acetone was identified on oxidation of I with chromic acid in acetic acid. On the basis of this evidence and the spectral data, two possible structures, 3-(3′-methyl-2′-butenylidene)-2-indolinone (Ia) or 2-(3′-methyl-2′-butenylidene)-3-indolinone (Ib) were considered for I.

The catalytic hydrogenation of I using Adams' catalyst gave a colorless viscid oil (IV), whose PMR spectrum exhibited, in addition to peaks arising from the protons of a 2-methylbutyl group and those of a 1,2-disubstituted benzene ring, a triplet at δ 3.40 attributable to a methine proton introduced during the hydrogenation. The chemical shift of this triplet was very similar to that of the signal due to the benzylic methine proton appearing in the PMR spectrum of α-phenylpropionic acid,[5] while the methine proton of tryptophan appears at δ 4.5 in its spectrum,[6] indicating that 3-(3′-methylbutyl)-2-indolinone (IVa) derived from Ia was a more likely structure for IV than 2-(3′-methylbutyl)-3-indolinone (IVb) formed from Ib.

Further evidence for this structural assignment was provided by the facts that the UV spectrum of IV $\lambda_{\max}^{\text{ethanol}}$ nm (log ε): 209 (4.395), 250 (3.968), 279 sh (3.208) was extremely similar to that of 3-methyl-2-indolinone, $\lambda_{\max}^{\text{ethanol}}$ nm (log ε): 207 (4.437), 249 (3.940), 278 sh (3.158),[7] whereas 3-indolinone derivatives have the UV spectrum, $\lambda_{\max}^{\text{ethanol}}$ nm (log ε): 230 (4.439), 256 sh (3.778), 400 (3.397,[8] entirely different from those of 2-indolinones. Thus, the structure of I was established as 3-(3′-methyl-2′-butenylidene)-2-indolinone (Ia).

Compound II, mp 213—214 °C, was assigned the molecular formula $C_{13}H_{13}NO$ on the basis of analytical and mass spectral data. II was insoluble in aqueous hydrochloric acid and gave no reaction with Dragendorff's test solution. The IR spectrum of II was suggestive of the presence of NH, lactam carbonyl groups and an aromatic ring, and the UV spectrum of II showed absorption maxima at 268 nm, 338 nm and 379 nm. The PMR spectrum of II showed signals assignable to $=C(CH_3)_2$ (δ 2.05, s), =CH–CH= (δ 6.85, 7.60, d, $J=9$ Hz), NH (δ 8.1, br. s) and four aromatic protons (δ 7.0—7.55, m). These chemical properties and spectral data suggested that the structure of II is analogous to that of I. II was concluded to be a geometrical isomer of I since heating of I with dilute alkali in ethanolic solution yielded II, resulting in the formation of a mixture of the two compounds. The reverse reaction from II was also observed to a minor extent, and hydrogenation and ozonolysis of II gave products identical with those formed from I. Furthermore, an ethanolic solution of II on standing deposited a barely soluble colorless solid, while the formation of this substance from I was not observed under the same conditions. In view of the fact that 3-methylene-2-indolinone is known to undergo dimerization by Diels-Alder reaction to form a colorless solid in various solvents,[7] the colorless product from II could be regarded as a dimer formed by an analogous process. It seemed that in the case of I the conformation of the 3-methyl-2-butenylidene side chain interfered sterically with the dimerization.

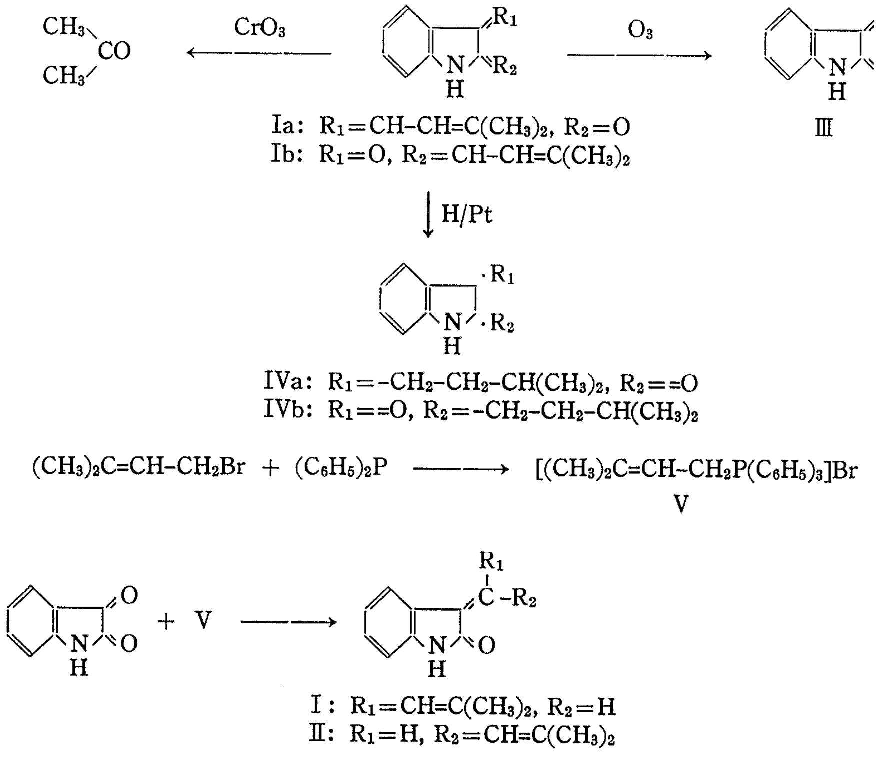

Chart 1

Table I. Crystal Data

Molecular formula	$C_{13}H_{13}ON$
Mr	199.3
Crystal system	Triclinic
Space group	$P\bar{1}$
Cell constant	
a	8.140(1) Å
b	13.134(2)
c	5.255(1)
α	92.51(4)°
β	103.73(4)
γ	77.89(4)
Volume	533.6(5) $Å^3$
Z	2
D_m	1.239(1) $g\cdot cm^{-3}$
D_x	1.240

Table II. Final Atomic Parameters with Their Standard Deviations ($\times 10^4$)

Atom	x	y	z	B_{11}	B_{22}	B_{33}	B_{12}	B_{13}	B_{23}
C 1	4311(6)	1245(4)	977(9)	144(9)	43(3)	242(19)	−28(9)	−19(21)	−40(12)
C 2	5618(8)	892(4)	3130(10)	142(11)	42(4)	222(22)	−31(10)	−11(24)	−32(14)
O 2	6498(6)	21(3)	3434(8)	189(9)	44(3)	279(17)	−14(8)	−102(20)	−45(11)
C 3	5728(8)	1811(6)	4942(10)	142(11)	43(4)	232(22)	−44(10)	27(25)	−44(14)
C 4	4034(8)	3745(5)	4074(12)	159(12)	47(4)	334(27)	−25(11)	62(29)	−43(16)
C 5	2749(9)	4407(5)	2218(14)	188(13)	48(4)	421(31)	−24(12)	44(33)	−5(18)
C 6	1990(9)	3980(5)	−178(14)	164(13)	59(5)	392(30)	−33(12)	8(31)	24(19)
C 7	2412(8)	2932(5)	−730(12)	133(11)	55(4)	309(25)	−15(11)	32(27)	−11(16)
C 8	3659(7)	2308(4)	1150(11)	130(10)	47(4)	235(22)	−45(10)	48(24)	−36(14)
C 9	4478(7)	2696(5)	3569(11)	133(11)	48(4)	232(22)	−53(10)	48(25)	−42(15)
C 10	6927(8)	1684(5)	7254(11)	152(12)	56(4)	212(22)	−61(11)	0(25)	−26(15)
C 11	7218(8)	2489(5)	9162(11)	160(12)	54(4)	222(22)	−64(11)	19(26)	−43(15)
C 12	8516(8)	2349(5)	11374(11)	148(11)	60(4)	246(23)	−62(11)	28(26)	−23(16)
C 13	8700(9)	3260(5)	13217(12)	224(15)	60(5)	270(26)	−98(14)	−31(31)	−63(17)
C 14	9796(9)	1347(6)	12193(14)	173(14)	68(5)	407(32)	−10(13)	−100(33)	6(20)

The temperature factors are:
$T=\exp[-(B_{11}h^2+B_{22}k^2+B_{33}l^2+2B_{12}hk+2B_{13}hl+2B_{13}hl+2B_{23}kl)]$.

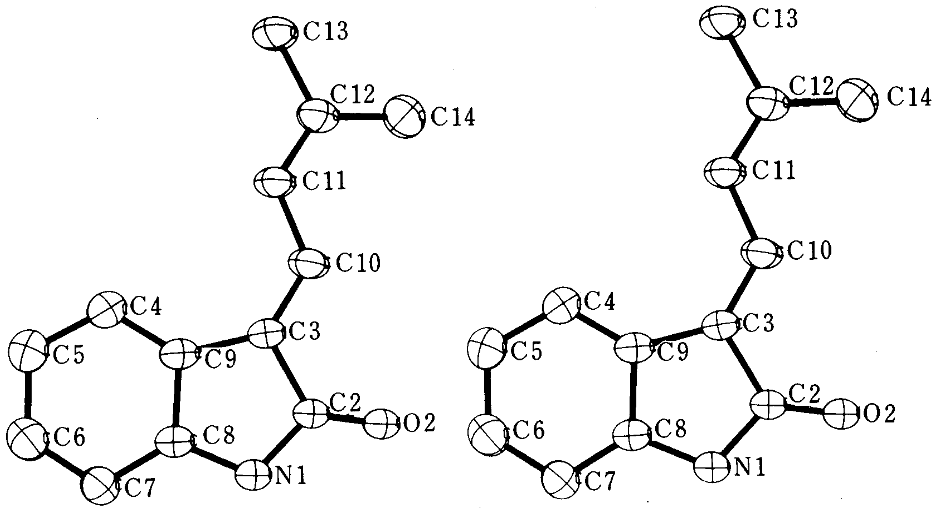

Fig. 1.

The structures of I and II were also confirmed by the following synthesis. Treatment of isoprene with hydrobromic acid provided γ,γ-dimethylallylbromide, which was then treated with triphenylphosphine to afford γ,γ-dimethylallylphosphonium bromide(V). Condensation of V with isatin in the presence of sodium methoxide in methanol gave two compounds, which were identical with I and II.

Determination of the geometric configuration of I was accomplished by X-ray diffraction analysis.

Recrystallization of I from hexane–ethyl acetate mixture gave well-formed needle crystals. The crystal and refinement data are summerized in Table I. The intensity data were collected on a Rigaku automated four-circle diffractometer using Cu $K\alpha$ radiation monochromated by means of a graphite plate, the ω-2θ scan mode being employed. 1659 independent reflections below $2\theta=130$ ° (F_o) $3\delta(F_o))$ were collected and used for the structure determination. Corrections were made for Lorentz and polarization effects, but not for absorption. The crystal structure was solved by the direct method with the MULTAN program,[9] and was refined by the block-diagonal least-squares method. The final R-value was 0.101 without hydrogen atoms. The atomic scattering factors for all atoms were taken from the literature.[10]

All numerical calculations were carried out on an ACOS-700 computer at the Computation Center of Osaka University using the UNICS program.[11]

The final atomic parameters are listed in Table II. A stereoscopic view of the molecular conformation is shown in Fig. 1. The chemical structure was confirmed as I (Chart 1). The 3-methyl-2-butenylidene side chain is E-connected to the indole ring with respect to the C(3)-C(10) bond. The torsion angle of C(2)-C(3)-C(10)-C(11) is 178.5(6)°. The 2-butenylidene group is almost planar and parallel to the indole ring (the dihedral angle of their planes=7.0 (4)°). The bond legtnhs and angles are normal, as shown in Fig. 2. Their standard deviations range from 0.007 to 0.009 Å for lengths and 0.5 to 0.6 ° for angles.

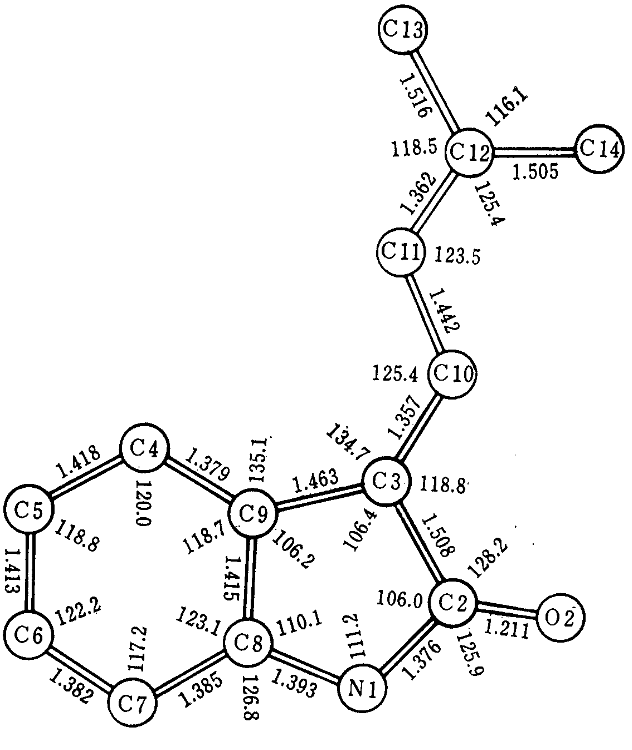

Fig. 2

Experimental

Isolation of the Compounds——The plant material (10 kg), obtained from the Tian-jin market was ground and extracted with three 30 l portions of EtOAc under reflux. The EtOAc solution was concentrated *in vacuo* to give a dark brown extractive (643 g). The extractive (250 g) was chromatographed over silica gel, eluted with hexane–EtOAc and the eluate was divided into the following fractions (500 ml/fraction): F 1—70 (3: 1), F 71—165 (2: 1), F 166—217 (1: 1) (figures in parentheses show the ratio of the solvents in v/v). The material from F 20—70 upon re-chromatography over alumina with hexane–EtOAc (2: 1) gave I and II. F 100—112 gave isoferulic acid. The material from F 186—217 upon re-chromatography over silica gel with $CHCl_3$–MeOH (10: 1) gave cimifugin.

(*E*)-3-(3′-Methyl-2′-butenylidene)-2-indolinone (I)——Recrystallized from hexane–EtOAc to give yellow needles, mp 200—203°C. *Anal.* Calcd for $C_{13}H_{13}NO$: C, 78.36; H, 6.58. Found: C, 78.06; H, 6.68. MS m/e: 199 (M^+). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 376 (3.78), 330 (4.36), 269 (4.11). IR ν_{max}^{Nujol} cm^{-1}: 3100 (NH), 1710

(C=O), 1620, 1600 (aromatic ring). PMR (in $CDCl_3$) δ ppm:[12] 2.07 (6H, s), 6.77 (1H, d, $J=12$ Hz), 6.9—7.6 (4H, m), 7.59 (1H, d, $J=12$ Hz), 8.33 (1H, br. s). Yield 0.015%.

(*Z*)-3-(3′-Methyl-2′-butenylidene)-2-indolinone (II)——Recrystallized from hexane–EtOAc to give yellow plates, mp 213—214°C. *Anal.* Calcd for $C_{13}H_{13}NO$: C, 78.36; H, 6.58. Found: C, 78.42; H, 6.51. MS *m/e*: 199 (M^+). UV $\lambda_{max}^{CHCl_3}$ (log ε): 379 (3.78), 338 (4.34), 268 (4.08). IR ν_{max}^{Nujol} cm^{-1}: 3150 (NH), 1700 (C=O), 1625, 1605 (aromatic ring). PMR (in $CDCl_3$) δ ppm: 2.05 (6H, s), 6.85 (1H, d, $J=9.0$ Hz), 7.0—7.55 (4H, m), 7.60 (1H, d, $J=9.0$ Hz), 8.10 (1H, br. s). Yield 0.006%.

Catalytic Hydrogenation of I, Formation of 3-(3′-Methylbutyl)-2-indolinone (IV)——Compound I (70 mg) was added to prereduced Adams' catalyst (PtO_2 30 mg) in EtOH (20 ml), and the mixture was stirred in the presence of hydrogen. After 15 ml of hydrogen had been taken up, the catalyst was filtered off and the filtrate was evaporated to dryness *in vacuo* to afford a colorless viscid oil (IV), 56 mg, MS *m/e*: 203 (M^+). UV λ_{max}^{EtOH} nm (log ε): 279 sh (3.208), 250 (3.968), 209 (4.395). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3200 (NH), 1700 (C=O), 1620 (aromatic ring). PMR (in DMSO-d_6) δ ppm: 0.84 (6H, d, $J=7.0$ Hz), 1.00—2.00 (5H, m), 3.40 (1H, t, $J=5.0$ Hz), 6.83—7.32 (4H, m), 10.40 (1H, br. s).

Catalytic Hydrogenation of II, Formation of 3-(3′-Methylbutyl)-2-indolinone (IV)——Compound II (70 mg) was added to prereduced Adams' catalyst (PtO_2 30 mg) in EtOH (20 ml), and the mixture was treated by the method described previously to afford IV.

Ozonolysis of I——I (90 mg) was ozonized in $CHCl_3$ (30 ml) under cooling (−7°C) and the solvent was evaporated off *in vacuo*. H_2O (50 ml) was added to the residue and the mixture was stirred, allowed to stand overnight, and extracted with EtOAc. The EtOAc solution was dried and concentrated *in vacuo*. The residue was recrystallized from a hexane–EtOAc mixture to afford III (43 mg), mp 198—199°C. The melting point showed no depression on admixture with an authentic sample of isatin. The IR and PMR spectra were identical with those of the authentic sample.

Oxidation of II with CrO_3, Formation of Acetone——II (50 mg) was dissolved in AcOH (1 ml), 15%-CrO_3–AcOH (1 ml) was added, and the whole was allowed to stand for 5 min at room temperature. The reaction mixture was diluted with H_2O (5 ml), neutralized with 5% $NaHCO_3$, gradually warmed on a water bath and exposed to filter paper which had been wetted with carbonyl reagent (2,4-dinitrophenylhydrazine reagent). The filter paper was extracted with MeOH and the MeOH solution was concentrated *in vacuo*. The residue was followed by TLC (silica gel, $CHCl_3$) and an orange spot corresponding to acetone-2,4-dinitrophenylhydrazone was detected.

Prenyl Bromide——A mixture of isoprene (25 g) and hydrobromic acid (60 g) was stirred at 0°C for 8 h, then allowed to stand overnight. The reaction mixture was neutralized with 15% Na_2CO_3 and extracted with ether. The ether solution was washed with water, dried, concentrated, then distilled *in vacuo* to give a colorless viscid oil (bp 51—53°C). Yield 11 g. PMR (in $CDCl_3$) δ ppm: 1.78, 1.72 (each 3H, s), 3.66 (2H, d, $J=9.0$ Hz), 5.50 (1H, t, $J=9.0$ Hz).

γ,γ-Dimethylallyltriphenylphosphonium Bromide (V)——A solution of triphenylphosphine (25 g) dissolved in dry benzene (30 ml) was cooled in ice-salt mixture, and prenyl bromide (10 g) was added. The mixture was allowed to stand at room temperature overnight. The precipitates were collected by filtration, washed with benzene and recrystallized from hexane–EtOAc to give a colorless crystalline powder, mp 239—240°C. Yield 27.2 g.

Formation of I and II——Compound V (4.12 g) was added to a solution of $NaOCH_3$ (0.54 g) in EtOH (20 ml) and the mixture was stirred for 15 min, then isatin (1 g) was added, and the whole was stirred for 30 min. The solvent was evaporated off, and the residue was extracted with EtOAc (100 ml) under reflux. The EtOAc solution was concentrated *in vacuo*, and the residue was purified by chromatography on alumina, using hexane–EtOAc (2: 1) as an eluent, to afford I (165 mg), mp 203—205°C and II (55 mg), mp 213—214°C.

Isoferulic Acid——Recrystallized from EtOAc to give colorless plates. mp 227—230°C (dec.). The melting point showed no depression on admixture with an authentic sample of isoferulic acid. The IR and PMR spectra were identical with those of the authentic sample. Yield 0.18%.

Cimifugin——Recrystallized from hexane–EtOAc to give colorless needles, mp 107—108°C. The melting point showed no depression on admixture with an authentic sample of cimifugin. The IR and PMR spectra were identical with those of the authentic sample. Yield 0.07%.

Acknowledgement The authors are grateful to Dr. T. Takano, Dr. Y. Morimoto, and their coworkers, Research Laboratory, Fujisawa Pharmaceutical Industries, for the mass spectra and microanalyses, and Dr. A. Numata of this college for the PMR spectra.

References and Notes

1) Preliminary report of this study: K. Hata, K. Baba, and M. Kozawa, *Chem. Pharm. Bull.*, **26**, 2279 (1978).
2) T. Inoue, C. Nakata, and K. Izawa, *Shoyakugaku Zasshi*, **24**, 76 (1970).
3) N. Sakurai, T. Inoue, and M. Nagai, *Yakugaku Zasshi*, **92**, 724 (1972).
4) M. Ito, Y. Kondo, and T. Takemoto, *Chem. Pharm. Bull.*, **24**, 580 (1976).

5) C.J. Pouchert and J.R. Campbell (eds.), "The Aldrich Library of NMR Spectra," Vol. 10, Aldrich Chemical Company, Inc., Milwaukee, 1974, p. 133.

6) C.J. Pouchert and J.R. Campbell (eds.), "The Aldrich Library of NMR Spectra," Vol. 8, Aldrich Chemical Company, Inc., Milwaukee, 1974, p. 68.

7) R.L. Hinman and C.P. Bauman, *J. Org. Chem.*, **29**, 2431 (1964).

8) A.I. Scott, "Interpretation of the Ultraviolet Spectra of Natural Products," Pergamon Press, Oxford, 1964, p. 175.

9) G. Germain, P. Main, and M.M. Woolfson, *Acta Cryst.*, **A27**, 368 (1971).

10) D.T. Cromer and J.T. Waber (edg.), "International Table for X-ray Crystallography," Vol IV, Kynoch Press: Birmingham, 1974, p. 71.

11) Universal Crystallographic Computing System (1973), Library of Programs, Computing Center of Osaka Univ.

12) The PMR spectra were measured by means of a Hitachi R-40 spectrometer (90 MHz) with TMS as an internal standard.