EXPERIMENTAL

Reagents.—Methionine (Eastman-Kodak, white label) was recrystallized from 40% ethanol. All other reagents were analytical grade. Water was purified by distilling tap distilled water from a potassium permanganate solution acidified with sulfuric acid.

The rate of oxidation of methionine with iodine and the rate of reduction of dehydromethionine by iodide forming iodine have been determined spectrophotometrically with a Cary model 11 or 15 by measuring the change in absorbance at 353 mμ, the absorption peak of I_3^-.

For the forward reaction, in general 5 ml. of a methionine solution was added to 45 ml. of a solution of buffer, potassium iodide, and iodine to yield 50 ml. of a solution with concentration as given for each figure. Very fast reactions were measured by injecting 1 ml. of methionine solution through the cover of the cell compartment into 2 ml. of solution containing buffer, iodide, and iodine while the instrument was operating.

For studying the reverse reaction, a stock solution of 0.025 *M* dehydromethionine was prepared by adding an equivalent amount of iodine and sodium hydroxide solution to a methionine solution, the pH was kept between pH 5 and 7. Iodide was removed with silver nitrate, the filtrate was brought to the calculated volume. Pseudo first-order rate constants were obtained as described above in the section for the reverse reaction.

The rate of hydrolysis of dehydromethionine was determined in 0.01 *M* solution of the cyclic species in the presence and in the absence of the buffer species. In both cases 10-ml. samples were withdrawn from the thermostated stock solution, 3 Gm. potassium iodide and 10 ml. 0.5 *N* hydrochloric acid were added, and the liberated iodine was titrated with 0.01 *N* sodium thiosulfate solution. From a plot of log (ml. 0.01 *N* $Na_2S_2O_3$) *versus* time, pseudo first-order rate constants were obtained.

Since dehydromethionine hydrolyzes under acid formation, the rate of hydrolysis in the absence of any buffer was carried out under simultaneous addition of sodium hydroxide solution. For this purpose the reaction solution was kept in a water jacket container closed with a ground-glass plate. Through the holes in the glass plate, electrodes and the tip of a microburet filled with 1 *N* sodium hydroxide solution were immersed into the solution stirred by a magnetic stirrer. Since for most cases a half-life of more than 10 min. was expected, the pH could be easily controlled manually.

In strong acidic solution the rate of hydrolysis was also determined spectrophotometrically by following a decrease in absorbance at 243 mμ.

REFERENCES

(1) Lavine, T. F., *J. Biol. Chem.*, **151**, 281(1943); Lavine, T. F., *Federation Proc.*, 4, 96(1945).
(2) "New and Nonofficial Remedies," J. B. Lippincott Co., Philadelphia, Pa., 1952, p. 627.
(3) "The National Formulary," 10th ed., J. B. Lippincott Co., Philadelphia, Pa., 1955, p. 378.
(4) "The National Formulary," 12th ed., Mack Publishing Co., Easton, Pa., 1965, p. 255.
(5) "Deutsches Arzneibuch 6. Ausgabe, Dritter Nachtrag," R. V. Decker's, Verlag-G. Schenck G.m.b.H, Hamburg-Berlin-Bonn, 1959, p. 116.
(6) Yada, H., Tanaka, J., and Nagakura, S., *Bull. Chem. Soc. Japan*, **33**, 1660(1960).
(7) Tamres, M., and Searles, S., Jr., *J. Phys. Chem.*, **66**, 1099(1962).
(8) Higuchi, T., and Gensch, K.-H., *J. Am. Chem. Soc.*, to be published.

Coumarins IV

Coumarins of *Pteryxia terebinthina*. Structures of Two New Coumarins, Isopteryxin and Calipteryxin

By B. EICHSTEDT NIELSEN* and T. O. SOINE

The ether extract of the root of *Pteryxia terebinthina* var. *californica* (Coult. and Rose) Mathias, in addition to osthol, anomalin (I), and pteryxin (III), afforded two new coumarins: 3′(*S*),4′(*S*)-3′-angeloyloxy-4′-acetoxy-3′,4′-dihydroseselin (IV) and 3′(*S*),4′(*S*)-3′-angeloyloxy-4′-senecioyloxy-3′,4′-dihydroseselin (V). These coumarins are provisionally named isopteryxin and calipteryxin, respectively.

In keeping with an interest in naturally occurring coumarins (1) and prompted by the recent isolation of anomalin (I) from *Angelica anomala* Lall by Hata *et al.*, and its structure determination as the 3′,4′-diangelate ester of 3′,4′-dihydroxy-3′,4′-dihydroseselin (II), the present paper reports the isolation of I and several related coumarins from *Pteryxia terebinthina* var. *californica* (Coult. and Rose) Mathias.[1] In particu-

Received July 5, 1966, from the Department of Pharmaceutical Chemistry, College of Pharmacy, University of Minnesota, Minneapolis.
Accepted for publication August 29, 1966.
Presented to the Divisional Meeting, Section 8, No. 4, Eleventh Pacific Science Congress, Tokyo, Japan, August 1966.
* The Royal Danish School of Pharmacy, Copenhagen, Denmark. Recipient of a grant from the Danish Government Fund for Scientific and Industrial Research for research at the University of Minnesota.
This investigation was supported by grant HE 07101 from the National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

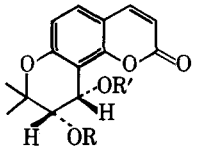
[1] The authors are indebted to Dr. T. G. Call, California State Polytechnic College, San Luis Obispo, Calif., for collection and identification of the plant material. Preliminary experiments by Dr. Call had shown the presence of osthol and a new coumarin which has since been identified as isopteryxin. Suitable specimens of the plant material have been placed in the herbarium of the Botany Department, University of Minnesota.

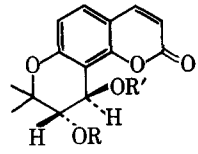
lar, in addition to the isolation of I, pteryxin (III), and osthol, two new coumarins, provisionally named isopteryxin (IV) and calipteryxin (V),[2] have been obtained. Spectral evidence further indicates the probability that a coumarin fraction, thus far unresolved, consists of approximately equal parts of lomatin angelate and senecioate or their isomers.

DISCUSSION

The structure of I, determined prior to the communication of Hata *et al.*, was elucidated by treatment of the isolated coumarin with methanolic potassium hydroxide to yield (+)-*cis*- and (−)-*trans*-methylkhellactones (VI and VII). The acidic hydrolytic component was shown to be only angelic acid by conversion to the *p*-phenylphenacyl ester. The conclusion that I was the 3′,4′-diangeloyloxy derivative of 3′,4′-dihydroseselin was substantiated completely by the nuclear magnetic resonance (NMR) spectrum which, in turn, was in complete accord with that described by Hata *et al.* (2) for I. Similarly, all physical constants were virtually identical, providing a secure basis for assuming the identity of the two coumarins from different sources.

The structure of V was ascertained by alkaline hydrolysis in dioxane to yield the (+)-*cis*- and (−)-*trans*-khellactones (VIII and IX), together with angelic and acetic acids identified by conversion to the *p*-phenylphenacyl ester derivatives. The NMR spectrum showed the 4 and 3 protons, respectively, as the usual pair of doublets at τ 2.38 and τ 3.79 (J = 9.5 c.p.s.) and *ortho* protons in the benzene ring as a pair of doublets at τ 2.62 and τ 3.20 (J = 8.5 c.p.s.). Other signals were noted as doublets (1H each) at τ 3.42 and τ 4.61 (J = 5 c.p.s.), at τ 3.87 (1H, multiplet), at τ 8.55 and τ 8.57 (singlets, 3H each), a multiplet centering at about τ 8.07 (6H), and a singlet at τ 7.90 (3H). The doublets at τ 3.42 and 4.61 are characteristic of the chemical shifts and pattern found for the 4′ and 3′ protons, respectively, in the diesters of *cis*-khellactone (3). The angelate character of one of the ester moieties is evident from the one-proton multiplet centering at τ 3.87 and the six-proton multiplet centering at about τ 8.07, whereas the three-proton singlet at τ 7.90 is characteristic of acetate methyl. The *gem*-dimethyl character of the compound at the 2′-position is evident from the pair of three-proton singlets at τ 8.55 and 8.57. With very minor differences, this spectrum was identical with that of pteryxin (III), the known *cis*-3′-acetoxy-4′-angeloyloxy-3′,4′-dihydroseselin (4). Thus, the weight of evidence indicates that IV is the correct structure. To confirm the placement of the angelate moiety at the 3′-position, advantage was taken of the well-known fact (4,5) that the ester moiety in the benzylic 4′-position is exceedingly labile under fixed base hydrolytic conditions, whereas the ester moiety at the 3′-position is much less reactive. Furthermore, the ester group at the 4′-position undergoes solvolytic *O*-alkyl cleavage and, finally, incorporation of the solvent into the system. Thus, with alcoholic base saponification, the final group in the 4′-position is determined by the alcohol, *i.e.*, methoxy, ethoxy, *etc.* When IV was subjected to ethanolic potassium hydroxide saponification for a short time the workup of the product provided small amounts of *cis*- and *trans*-ethylkhellactones (X and XI) but, more important, also yielded a large quantity of a noncrystalline blue fluorescent compound (XII), apparently identical to the compound obtained by Hata *et al.* (2) in a similar fashion from anomalin (I). The NMR spectrum of this compound showed the usual pattern, integration and splitting constants for the 3,4,5, and 6 protons of the parent compound and showed the usual multiplet pattern and integration expected for the angelate methyls and vinyl proton of a single angelate moiety. The acetate methyl of the parent spectrum was missing but the typical pattern for an ethoxy group was evident with the methyl protons being observed as a triplet at τ 8.67 (3H, J = 7.5 c.p.s.) and the methylene protons as a quartet at τ 5.93 (2H, J = 7.5 c.p.s.). The doublets corresponding to the 3′ and 4′ protons were now found at τ 4.80 and 5.50 (J = 2 c.p.s.) indicating the *trans*-configuration expected on the basis of reasons discussed in an earlier paper (4). These results provide strong evidence for the structure of isopteryxin as 3′-angeloyloxy-4′-acetoxy-3′,4′-dihydroseselin.





I, R = R′ = Angeloyl
II, R = R′ = H
III, R = Acetyl; R′ = Angeloyl
IV, R = Angeloyl; R′ = Acetyl
V, R = Angeloyl; R′ = Senecioyl
VI, R = H; R′ = CH_3
VIII, R = R′ = H
X, R = H; R′ = C_2H_5

VII, R = H; R′ = CH_3
IX, R = R′ = H
XI, R = H; R′ = C_2H_5
XII, R = Angeloyl; R′ = C_2H_5

The structure of V was established by a short hydrolysis of the compound with ethanolic potassium hydroxide which yielded principally XII, unreacted V, and small amounts of X and XI. The acidic hydrolytic components were identified as principally senecioic acid and, in much smaller amount, angelic acid through the *p*-phenylphenacyl esters. The NMR spectrum of V was remarkably similar to that of I with the major difference being the signals from the senecioyl group at τ 8.05 and 7.78 (3H each) and τ 4.37 (1H). In addition, the signals from the angelate moiety integrated for only one-*half* the values found for I. The decision as to the location of the senecioyl group at 4′ was obvious from the isolation of XII, identical to that obtained from IV in the same way. These results provide conclusive

[2] Hata *et al.* (9) have recently reported on a new coumarin, peuformosin, from the root of *Peucedanum formosanum* Hayata which appears to be structurally identical to calipteryxin. Peuformosin, inexplicably, has a somewhat higher melting point (155–156°) than calipteryxin but has a roughly equal {$[\alpha]D^{27}$ + 67.3° ($CHCl_3$)}, although opposite rotation. Since they have chosen to utilize a name indicating its botanical origins we choose to retain the name calipteryxin to identify this enantiomeric form and thus to preserve its botanical origin. If peuformosin, as seems likely, is the mirror image of calipteryxin it is possible to assign the 3′(*R*), 4′(*R*) 3′-angeloyloxy-4′-senecioyloxy-3′,4′-dihydroseselin nomenclature to it.

evidence for the structure of calipteryxin as 3′-angeloyloxy-4′-senecioyloxy-3′,4′-dihydroseselin.

The NMR spectra of I, IV, and V all show the same coupling constant of $J_{3',4'}$ = 5.0 c.p.s. Furthermore, the signals arising from the two methyl groups in the chroman ring differ by only 0.02 p.p.m. As pointed out by Lemmich *et al.* (6) these spectral features are typical of khellactone derivatives possessing the relative *cis*-configuration. From the observation of a $J_{3',4'}$ = 2.0 c.p.s. and of well-separated *gem*-dimethyl signals (Δ = 0.7 p.p.m.) in the NMR spectrum of XII it is, in turn, quite safe to deduce the relative *trans*-configuration for this compound, bearing out the expectations based on chemical grounds.

The absolute configuration of all previously isolated diesters of 3′,4′-dihydroxy-3′,4′-dihydroseselin has been shown to be 3′(*S*),4′(*S*) (4,7). Since the alkaline degradation products from anomalin (I) and isopteryxin (IV) are shown to be mixtures, respectively, of VI and VII and of VIII and IX the configuration 3′(*S*) may be assigned to I and IV. By optical comparison of XII obtained from IV with that obtained from V the configuration 3′(*S*) may be assigned to calipteryxin (V) also. Since I, IV, and V are all of the *cis*-configuration it follows, therefore, that they all possess the configuration 3′(*S*), 4′(*S*).

EXPERIMENTAL

Melting points were determined in capillary tubes in a Thomas-Hoover melting point apparatus checked for accuracy against a set of standard samples. Infrared spectra were determined on a Perkin-Elmer 237B grating infrared spectrophotometer or a Perkin-Elmer spectrophotometer, model 21. Values of [α] were determined on a Perkin-Elmer 141 polarimeter. NMR spectra were determined on a Varian Associates A-60 instrument through the courtesy of Dr. William B. Schwabacher, School of Chemistry, University of Minnesota. Analyses were performed by the Microanalytical Laboratory, School of Chemistry, University of Minnesota, or by A. Bernhardt, Mülheim, Germany.

Material.—Dried roots of *P. terebinthina* var. *californica* (Coult. and Rose) Mathias, collected at an elevation of 7200 ft. on June 26, 1965, in basalt rocks the first mile after leaving Slinkard Creek and ascending toward Monitor Pass, Alpine County, Calif.

Isolation of the Coumarins.—The dried and ground root (500 Gm.) was extracted with diethyl ether. Upon evaporation of the solvent, 88 Gm. of a viscous oil remained. This residue was dissolved in 90% methanol, defatted with petroleum ether, and evaporated. The residue (60 Gm.) was chromatographed on silica gel (J. T. Baker Co., No. 3405, 450 Gm.) activated at 120° and impregnated with 10% of water. Upon elution with benzene, benzene–chloroform, and, subsequently, chloroform–ethyl acetate, the following fractions were collected.

(*A*) A blue-fluorescent, noncrystalline material (71 mg.). Thin-layer chromatography on silica gel (Merck) using different solvent systems gave only one spot with the same R_f value as jatamansin.[3] The NMR spectrum, however, indicated that it was a mixture of two monoesters, possibly lomatin angelate (*i.e.*, jatamansin) and the isomeric senecioate. The fraction has not been investigated further.

(*B*) Osthol, 10.2 Gm., m.p. 84.5–85.0°. This was identified by its infrared and NMR spectra and also by a mixed melting point determination with an authentic sample of osthol.

(*C*) A fraction (1.3 Gm.) which was rechromatographed on silica gel (J. T. Baker Co., No. 3405, 100 Gm.) activated at 120° and impregnated with 3% of water. The eluant was a mixture of methylene chloride–carbon tetrachloride (2:1) to which increasing amounts of ethyl acetate were added. The following substances were eluted: (*a*) 307 mg. of a blue-fluorescent compound (I), recrystallized from ether, m.p. 173.0–174.0°, $[\alpha]_D^{25}$ −48.5° (*c* 0.5, chloroform), $[\alpha]_{546}^{25}$ − 53° (*c* 0.5, chloroform).

Anal.—Calcd. for $C_{24}H_{26}O_7$: C, 67.59; H, 6.15. Found: C, 67.87; H, 6.15.

(*b*) A 314-mg. quantity of a blue-fluorescent compound (V), recrystallized from ether, m.p. 147.5–148.0°, $[\alpha]_D^{25}$ −55° (*c* 0.8, chloroform), $[\alpha]_{546}^{25}$ −62° (*c* 0.8, chloroform).

Anal.—Calcd. for $C_{24}H_{26}O_7$: C, 67.59: H, 6.15. Found: C, 67.55; H, 6.06.

(*D*) Further elution yielded a fraction (5.8 Gm.) which was rechromatographed using the same conditions as mentioned for fraction (*C*). The following substances were obtained: (*a*) 130 mg. of a blue-fluorescent compound, m.p. 86.0–86.5°, $[\alpha]_D^{23}$ +12.5° (*c* 2.0, ethanol, 95%), $[\alpha]_D^{25}$ − 4° (*c* 1.1, chloroform). The infrared spectrum was identical with that of an authentic sample of pteryxin (III).

(*b*) A 3.5-Gm. quantity of a blue-fluorescent compound (IV), recrystallized from ethanol and cyclohexane, m.p. 135.0–135.5°, $[\alpha]_D^{24}$ − 39° (*c* 2.0, ethanol, 95%), $[\alpha]_D^{25}$ −45° (*c* 1.3, chloroform), $[\alpha]_{546}^{25}$ −52° (*c* 1.3, chloroform).

Anal.—Calcd. for $C_{21}H_{22}O_7$: C, 65.27; H, 5.74. Found: C, 65.29; H, 5.82.

(*E*) Elution with ethyl acetate to which increasing amounts of methanol were added yielded a mixture of several blue-fluorescent compounds. This mixture is currently under investigation.

Treatment of I with Methanolic Potassium Hydroxide.—Compound I (175 mg.) was dissolved in 10 ml. of methanol containing 1.5 Gm. of potassium hydroxide and the mixture refluxed for 6 min. Upon cooling, the reaction mixture was acidified with 4 *N* sulfuric acid, water (20 ml.) was added, and after standing for 30 min. adjusted to pH 8 with sodium carbonate and finally extracted with chloroform. The chloroform extract, after drying and evaporation, was chromatographed on silica gel (Merck, 20 Gm.) activated at 120° and impregnated with 5% of water. The eluant was benzene to which increasing amounts of ethyl acetate were added. The following substances were obtained: (*a*) 4 mg. (+)-*cis*-methyl khellactone (VI), recrystallized from petroleum ether, m.p. 126.5–127.0°, $[\alpha]_D^{25}$ + 74° (*c* 0.15, chloroform), $[\alpha]_{546}^{25}$ + 90° (*c* 0.15, chloroform). The infrared spectrum was identical with that of an authentic sample of (+)-*cis*-methyl khellactone. (*b*) A 74-mg. quantity of (−)-*trans*-methyl khellactone (VII), recrystallized from ether–petroleum ether, m.p. 162.5°, $[\alpha]_D^{25}$ − 30° (*c* 1.0, chloroform). The infrared spectrum was identical with that of an authentic sample of (−)-*trans*-methyl khellactone.

The aqueous phase (pH 8) was evaporated, acidified with 4 *N* sulfuric acid, and extracted with ether.

[3] Supplied by Dr. S. C. Bhattacharyya, National Chemical Laboratory, Poona, India.

TABLE I.—OPTICAL ROTATIONS OF COMPOUND XII OBTAINED FROM COMPOUNDS IV AND V

Compd. from	Concn. in $CHCl_3$	$[\alpha]_D^{25}$	$[\alpha]_{578}^{25}$	$[\alpha]_{546}^{25}$	$[\alpha]_{436}^{25}$	$[\alpha]_{364}^{25}$
IV	0.4	−10.2	−9.6	−9.3	+15.4	+69.2
V	0.2	−11.1	−10.0	−10.0	+11.7	+75.9

Upon addition of dicyclohexylethylamine (100 μl.) the dried ether extract was evaporated. The residue was converted to the *p*-phenylphenacyl ester according to the method described by Stodola (8). A chloroform extract, containing a mixture of this ester and unreacted *p*-phenylphenacyl bromide, was evaporated and chromatographed on silica gel (Merck, 10 Gm.), activated at 120°, and impregnated with 10% of water. The eluant was benzene–petroleum ether (2:1) to which increasing amounts of benzene were added. *p*-Phenylphenacyl angelate (92 mg.), m.p. 88.5–89.0°, was obtained. The identity was established by infrared spectroscopy and by mixed melting point determination with an authentic sample.

Treatment of IV with 1 N Potassium Hydroxide.—A solution of IV (500 mg.) in 5 ml. of dioxane and 15 ml. of 1 *N* potassium hydroxide was left at room temperature overnight. The reaction mixture was acidified with 2 *N* sulfuric acid and, after standing for 45 min., was adjusted to pH 8 with sodium carbonate and extracted with chloroform. The dried extract was evaporated and the residue chromatographed on silica gel (J. T. Baker Co., No. 3405, 15 Gm.) activated at 120° and impregnated with 10% of water. The eluant was chloroform to which increasing amounts of ethyl acetate were added. Besides unreacted IV (81 mg.) the following compounds were obtained: (*a*) 60 mg. (+)-*cis*-khellactone (VIII), recrystallized from benzene, m.p. 172.0–173.0°, $[\alpha]_D^{22}$ + 80° (*c* 0.5, chloroform). The identity was established by infrared spectroscopy. (*b*) A 96-mg. quantity of (−)-*trans*-khellactone (IX), recrystallized from benzene–chloroform, m.p. 184.5–186.0°, $[\alpha]_D^{22}$ − 17.5° (*c* 1.1, chloroform). The identity was established by infrared spectroscopy.

The aqueous phase (pH 8) when worked up as described above for compound I yielded *p*-phenylphenacyl angelate, m.p. 87.5–89.0°, and *p*-phenylphenacyl acetate, m.p. 110.5–111.0°.

Treatment of IV with Ethanolic Potassium Hydroxide.—A solution of IV (300 mg.) in 12 ml. of ethanol was mixed with 12 ml. of 1 *N* ethanolic potassium hydroxide, left at room temperature for 45 sec., and acidified with 4 *N* sulfuric acid. Upon standing for 45 min., 30 ml. of water was added and the mixture extracted with chloroform. The dried extract was evaporated and chromatographed on silica gel (Merck, 20 Gm.) activated at 120° and impregnated with 5% of water. The eluant was methylene chloride–carbon tetrachloride (2:1). XII (221 mg.) was obtained. Further elution with methylene chloride–carbon tetrachloride (2:1) to which increasing amounts of ethyl acetate were added yielded small amounts of (+)-*cis*- and (−)-*trans*-ethylkhellactones (X and XI) which were identified by co-chromatography with authentic samples.

Treatment of V with Ethanolic Potassium Hydroxide.—A solution of V (169 mg.) in 8 ml. of ethanol was mixed with 8 ml. of 1 *N* ethanolic potassium hydroxide, left at room temperature for 1 min., acidified with 4 *N* sulfuric acid, and then diluted with 25 ml. of water. Upon standing for 30 min. the mixture was adjusted to pH 8 with sodium carbonate and extracted with chloroform. The extract, after drying and evaporation, was chromatographed as described above for IV. XII (78.5 mg.) was obtained and, as in the previous case, was found to be an uncrystallizable oil. Further elution yielded 60.7 mg. of unreacted V and small amounts of X and XI (identified by co-chromatography with authentic samples). The water phase (pH 8) was worked up as described for I to yield 0.8 mg. of *p*-phenylphenacyl angelate and 26 mg. of *p*-phenylphenacyl senecioate, m.p. 143.5–145.0°. The identity of these esters was established by infrared comparison with authentic samples.

Comparison of Optical Activity of XII from IV and V.—The noncrystalline compound XII obtained from isopteryxin (IV), distilled at 0.1 mm. Hg (160–180°) when compared with the same compound from calipteryxin (V) obtained directly by chromatography gave the values shown in Table I.

REFERENCES

(1) Soine, T. O., *J. Pharm. Sci.*, **53**, 231(1964).
(2) Hata, K., Kozawa, M., and Ikeshiro, Y., *Chem. Pharm. Bull.*, **14**, 94(1966).
(3) College of Pharmacy, University of Minnesota, Minneapolis, Minn., unpublished data.
(4) Willette, R. E., and Soine, T. O., *J. Pharm. Sci.*, **51**, 149(1962).
(5) Smith, E., Hosansky, N., Bywater, W. G., and van Tamelen, E. E., *J. Am. Chem. Soc.*, **79**, 3534(1957).
(6) Lemmich, J., Lemmich, E., and Nielsen, B. E., *Acta Chem. Scand.*, to be published.
(7) Schroeder, H. D., Bencze, W., Halpern, O., and Schmid, H., *Chem. Ber.*, **92**, 2338(1959).
(8) Stodola, F. H., *Microchem. J.*, **7**, 389(1963).
(9) Hata, K., Kozawa, M., and Yen, K., *Chem. Pharm. Bull.*, **14**, 442(1966).