Coumarins I

Isolation, Purification, and Structure Determination of Pteryxin and Suksdorfin

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Pteryxin and suksdorfin were isolated from petroleum ether extracts of *Pteryxia terebinthina* (Hook.) Coulter and Rose var. *terebinthina* and *Lomatium suksdorfii* (Wats.) Coulter and Rose, respectively. A suitable chromatographic procedure was developed for their purification. Upon hydrolysis, these coumarins yielded the same isomeric alcohols obtained from visnadin and samidin. In addition, an alcohol and its acetate which had not been reported previously were obtained and assigned the structures (+)-*cis*-ethylkhellactone and (−)-*cis*-acetylethylkhellactone. Pteryxin, during the hydrolysis, yielded angelic and acetic acids. Quantitative hydrolytic studies, optical rotations, isolation of the partially hydrolyzed coumarins as acetates, and ultraviolet and infrared absorption studies led to the establishment of the following structure for pteryxin: 3′-acetyl-4′-angeloyl-(+)-*cis*-khellactone; and for suksdorfin: 3′-acetyl-4′-isovaleryl-(+)-*cis*-khellactone. The probable conformation of these substances is discussed.

THE PRESENT investigations were stimulated by the recent interest in the isolation and characterization of various naturally occurring chromones and coumarins from *Umbelliferae* possessing certain and distinct pharmacological activities. The chromone khellin, which is obtained from the umbellifer *Ammi visnaga*, has had some clinical success (1). From the strongly vasodilatory oil which remains after the isolation of khellin, Smith and co-workers reported the isolation and structural determinations of the coumarins visnadin, samidin, and dihydrosamidin (2). Visnadin has undergone clinical studies under the registered name Provismine (3). The absolute configurations of these latter substances were established by Schmid and co-workers (4).

In an extensive pharmacognostical investigation of *Pteryxia terebinthina* (Hook.) Coulter and Rose var. *terebinthina*, Call (5) isolated a low-melting solid, which he named pteryxin, and which exhibited antispasmodic activity (6). Preliminary characterization studies by Pettinato (7) indicated pteryxin to be a coumarin containing two alkali-consuming groups in addition to the expected lactone grouping.

Call (8) isolated another crystalline substance from the fruit of *Lomatium suksdorfii* (Wats.) Coulter and Rose which he named suksdorfin A. He also showed that it possessed antispasmodic properties. A preliminary phytochemical study (9) and an analysis of the volatile oil content (10) of this plant have been reported.

The investigation reported here concerns the isolation, purification, and determination of structure of pteryxin and suksdorfin.

Detailed pharmacological studies will be reported at a later date.

EXPERIMENTAL

Melting points were determined in capillary tubes in an oil bath with a thermometer that read accurately for a set of standard samples. Values of $[\alpha]_D$ have been approximated to the nearest degree. Infrared spectra were determined on a Perkin and Elmer spectrophotometer, model 137. Ultraviolet spectra were determined in 95% ethanol on Cary recording spectrophotometers, models 11 and 14.

Material.—Dried roots of *Pteryxia terebinthina* (Hook.) Coulter and Rose var. *terebinthina*, collected at an elevation of 400 ft. near Rufus, Oreg., and dried fruits of *Lomatium suksdorfii* (Wats.) Coulter and Rose, collected at an elevation of 1,200 ft. near Klickitat, Wash., were ground in a Jacobsen laboratory mill to an approximately No. 20 powder in preparation for extraction.[1]

Isolation of Pteryxin (I) from *P. terebinthina*.—The ground root (1 Kg.) was washed with two 2-L. portions of ice-cold petroleum ether (b.p. 30–60°), which were discarded. The washed root was boiled with three 2-L. portions of Skellysolve B (b.p. 65–70°); the menstruum after each extraction was filtered into a large beaker. Upon cooling, a resinous, oily deposit separated, from which the clear liquor was decanted. The liquor became cloudy on pouring and, after standing overnight, was decanted

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[1] The authors are indebted to Dr. T. G. Call, 115 W. Rancho Rd., Corona, Calif., for collection and identification of the plant materials, and to Dr. G. B. Ownbey, Botany Department, University of Minnesota, Minn., for confirmation of the identities. Suitable specimens have been placed in the herbarium of the Botany Department, University of Minnesota.

from the oil deposit which again separated. After this process had been repeated several times, large tetragonal crystals formed on the bottom of the beaker. The crystals were collected in successive crops to yield 15.3 Gm. of crude pteryxin. The resinous, oily deposits were combined, dissolved in boiling Skellysolve B, and treated in the same manner. An additional 4.7 Gm. was obtained for a total of 20 Gm. (2%) of crude pteryxin. Recrystallization from Skellysolve B gave slightly yellow tetragonal prisms melting at 79–81°. The melting point was not depressed on admixture with a sample of pteryxin previously isolated by Call.

A twice recrystallized sample of pteryxin, dissolved in Skellysolve B, was spotted on several chromatostrips.[2] Each chromatostrip was developed in an ascending manner in a stoppered test tube containing about 2 ml. of solvent. Of the several solvent systems tried, only 10% anhydrous ethanol in Skellysolve B separated the sample into four fluorescent spots.

Twice recrystallized pteryxin (500 mg.) dissolved in anhydrous ethanol (5 ml.) was added to the top of a column of silica gel (150 Gm., Davison, commercial grade) prepared in a slurry with 10% anhydrous ethanol in Skellysolve B. Development with the same solvent separated the material into four zones which were eluted. Evaporation of the first two and last fractions gave only trace residues. The third fraction afforded a colorless resin (420 mg.), which was dissolved in boiling Skellysolve B. The solution was filtered, concentrated to about two-thirds its volume, and allowed to stand in a stoppered flask. After several days small colorless prisms began to form. The stopper was removed and the flask covered with a piece of filter paper. After four days, pteryxin crystallized as large, colorless, tetragonal prisms which were filtered off and dried at room temperature in vacuum for several days, m.p. 81.5–82.5°; $[\alpha]_D^{22}$ +10° (c 0.650, C_2H_5OH); ultraviolet spectrum (Fig. 1): $\lambda_{min.}$ 252 (log ϵ 3.57) and 263 mμ (3.20); $\lambda_{max.}$ 218 (4.31) sh., 244 (3.71) sh., 255 (3.60), 300 (3.92) sh., and 323 mμ (4.11); infrared spectrum (Nujol mull) (Fig. 2): 1757–1745 (α-pyrone and α,β-unsatd. ester C=O), 1637 (α-pyrone ring C=C), 1621, 1502 (aromatic C=C), 1587 (aromatic ring with conjugated C=C), 1321, 1107 (α,β-unsatd. ester C—O), 1122 (Ar—O—CR_3), 908 [$(CH_3)_2C$—O], 846 (1,2,3,4-aromatic substitution) and 839 cm.$^{-1}$ (trisubstituted ethylene C—H); (c 2.0, CH_2Cl_2): 1393 and 1377 cm.$^{-1}$ ([$CH_3)_2C$=]. [3] Development of it on a chromatostrip with 10% anhydrous ethanol in Skellysolve B gave a single homogeneous spot.

Anal.[4]—Calcd. for $C_{21}H_{22}O_7$: C, 65.27; H, 5.74; mol. wt., 386.39. Found: C, 66.18; H, 6.11;[5] mol. wt. (Rast), 339.

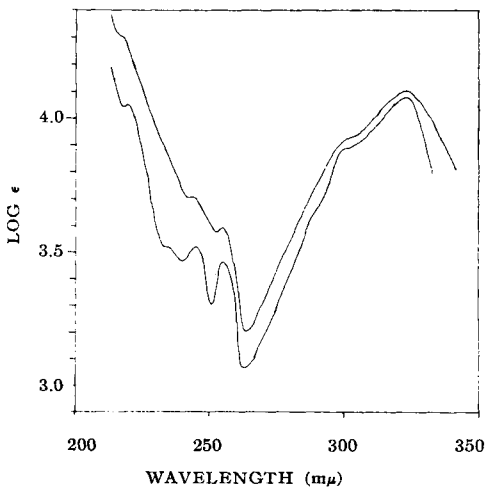


Fig. 1.—Ultraviolet absorption spectra in 95% ethanol. Upper curve, pteryxin; lower curve, suksdorfin.

Isolation of Suksdorfin (II) from *L. Suksdorfii*.—The ground fruit (500 Gm.) was extracted continuously for two days in a Soxhlet apparatus with Skellysolve B (b.p. 65–70°). The colorless crystalline deposit which formed in the concentration flask was filtered off, rinsed with Skellysolve B, and air-dried to give 6 Gm. (1.2%) of suksdorfin, m.p. 130–135°. Five recrystallizations from Skellysolve B afforded fine colorless needles (2 Gm.), m.p. 140–141°. The melting point was not depressed on admixture with a sample of suksdorfin A previously isolated by Call.

A sample of recrystallized suksdorfin was spotted on several chromatostrips which were developed as before, using various solvent systems. Only 30% acetone in Skellysolve B was found to separate a sample into three fluorescent spots.

Recrystallized suksdorfin (0.5 Gm.) was chromatographed on a column of silica gel (150 Gm., Davison, commercial grade) prepared in a slurry with Skellysolve B. Development was conducted with Skellysolve B to which acetone was added gradually (1% increase every 10 ml.) until a concentration of 30% acetone was reached. The major fraction was eluted; the column extruded; and the two remaining zones separated and eluted from the adsorbent with acetone, which was evaporated to give only faint traces. Two recrystallizations of the major fraction from Skellysolve B followed by drying at 100° in vacuum overnight afforded fine colorless needles, m. p. 140.5–141°; $[\alpha]_D^{24}$ + 4° (c 0.500, C_2H_5OH); ultraviolet spectrum (Fig. 1): $\lambda_{min.}$ 240 (log ϵ 3.47), 251 (3.30) and 263 mμ (3.07); $\lambda_{max.}$ 219 (4.05), 234 (3.52) sh., 245 (3.52), 255 (3.46), 300 (3.88) sh., and 323 mμ (4.07); infrared spectrum: (Nujol mull) (Fig. 3): 1754 (ester C=O), 1736 (α-pyrone C=O), 1631 (α-pyrone ring C=C), 1610, 1493, 1445 (aromatic C=C), 1572 (aromatic ring with conjugated C=C), 1250, 1120 (Ar—O—CR_3), 902 [$(CH_3)_2C$—O] and 856 cm.$^{-1}$ (1,2,3,4-aromatic substitution); (c 2.0, CH_2Cl_2): 1395 and 1376 cm.$^{-1}$ [$C(CH_3)_2$]. A sample developed with 30% acetone in Skellysolve B on a chromatostrip showed only one distinct spot. In a volatile acid

[2] The chromatostrips consist of glass strips, 13 × 136 mm., coated on one surface with a thin film of silicic acid containing 5% starch and 0.1% each of zinc silicate and zinc-cadmium sulfide. Their preparation and development procedure is essentially the same as described by Stanley (11).

[3] The assignment of groups responsible for some of the absorption bands in the infrared spectra of the compounds discussed in this paper are in agreement with the findings of Schroeder, *et al.* (4).

[4] Microanalyses were determined by the Microanalytical Laboratory of the School of Chemistry, University of Minnesota, Minneapolis, or by Drs. Weiler and Strauss, 164 Banbury Rd., Oxford, England.

[5] Average percentages. All attempts to prepare a sample which analyzed accurately were unsuccessful.

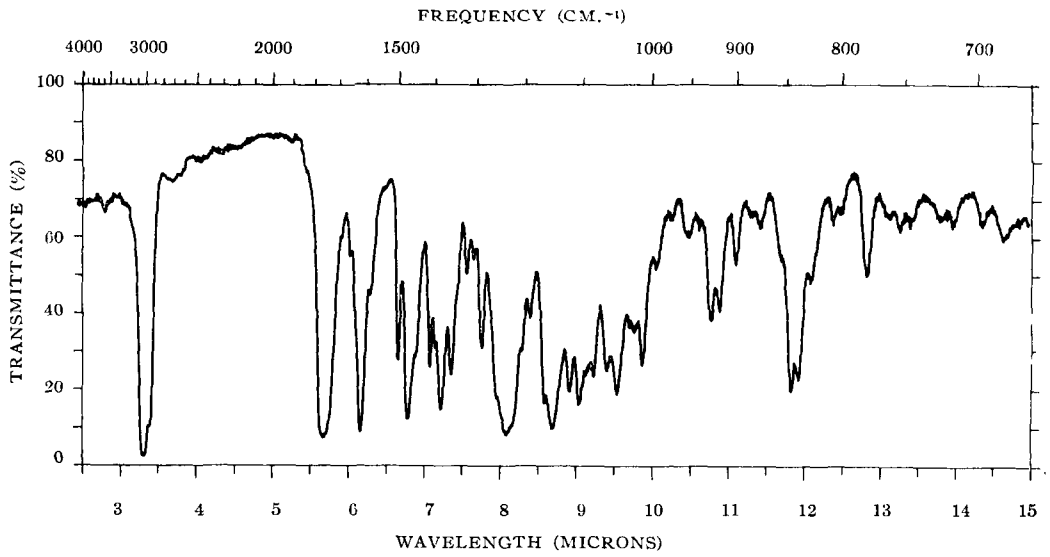


Fig. 2.—Infrared absorption spectrum of pteryxin (Nujol mull).

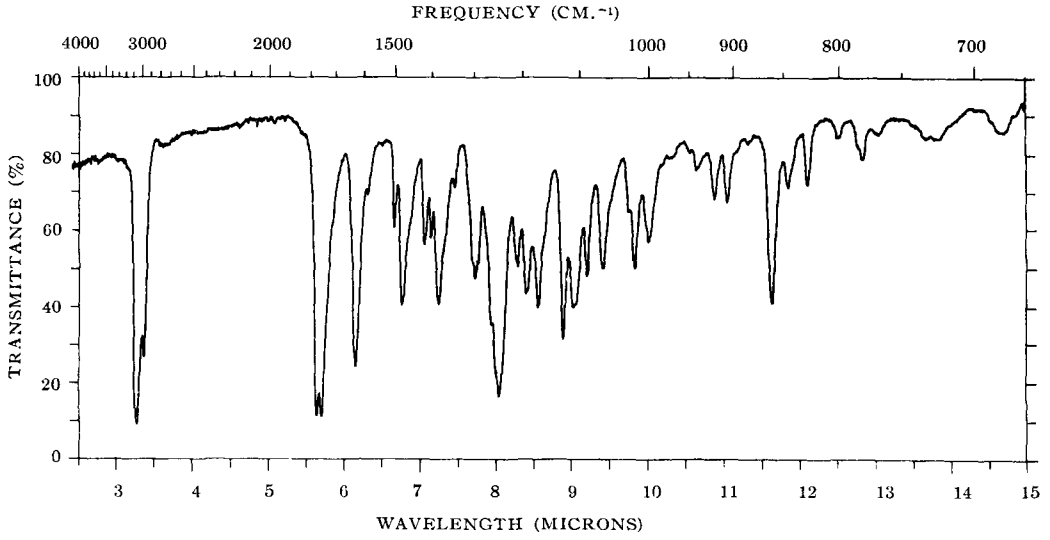


Fig. 3.—Infrared absorption spectrum of suksdorfin (Nujol mull).

determination suksdorfin yielded an amount of acid equivalent to 38.42 ml. of 0.01 *N* sodium hydroxide; calcd. for two acids, 41.89 ml.

Anal.—Calcd. for $C_{21}H_{24}O_7$: C, 64.95; H, 6.23; mol. wt., 388.30. Found: C, 64.88; H, 6.31; mol. wt. (Rast), 357.

Hydrogenation of Pteryxin.—Chromatographically pure pteryxin (77.2 mg., 0.2 mmole) was dissolved in ethanol (10 ml.) and hydrogenated at atmospheric pressure in the presence of platinum oxide (20 mg.). After 20 minutes, 0.17 mmole of hydrogen was consumed, the hydrogenation was interrupted, the catalyst filtered off, and the filtrate evaporated on a steam bath. Two crystallizations of the oily residue from dilute ethanol followed by drying at 78° in vacuum gave dihydropteryxin as fine colorless needles, m.p. 103–104°; ultraviolet spectrum: $\lambda_{min.}$ 262 mμ (log ε 3.20); $\lambda_{max.}$ 218 (4.23) and 322 mμ (4.15); infrared spectrum (Nujol mull): 1172 cm.$^{-1}$ (satd. ester C—O).

Anal.—Calcd. for $C_{21}H_{24}O_7$: C, 64.95; H, 6.23. Found: C, 65.09; H, 6.04.

Chromatographically pure pteryxin (77.2 mg., 0.2 mmole) was treated as above. After 3 hours, 0.36 mmole of hydrogen was consumed, the hydrogenation interrupted, and the product worked up as above. Recrystallization from dilute ethanol and drying for several hours at 100° in vacuum afforded tetrahydropteryxin as fine colorless needles, m.p. 115–115.5°; ultraviolet spectrum: $\lambda_{min.}$ 262 (log ε 2.92) and 301 mμ (3.64); $\lambda_{max.}$ 243 (3.50) sh., 255 (3.22), 284 (3.72) sh., 291 (3.76), and 323 mμ (3.82); infrared spectrum (Nujol mull): 1764 (γ,δ-unsatd. δ-lactone C=O) and 1739 cm.$^{-1}$ (satd. ester C=O).

Anal.—Calcd. for $C_{21}H_{26}O_7$: C, 64.60; H, 6.71. Found: C, 64.21; H, 6.64.

Ethanolic Alkaline Hydrolysis.—Crude pteryxin (15 Gm.), dissolved in 95% ethanol (150 ml.), was mixed with 1 *N* ethanolic sodium hydroxide (150 ml.) and allowed to stand at room temperature for 30 minutes. The mixture was poured into water (2.5 L.), acidified with concentrated sulfuric acid (4 ml.), and extracted with six 500-ml. portions of ether. The combined ether extract was backwashed with three 200-ml. portions of 5% sodium carbonate solution, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue crystallized from dilute ethanol as colorless needles (4.1 Gm.). A sample of it separated into five distinct fluorescent spots on a chromatostrip developed with 50% ethyl acetate in Skellysolve B. Recrystallization from dilute ethanol gave colorless needles (2.5 Gm.) which separated into three spots on a chromatostrip. A portion of these needles (1.0 Gm.) was chromatographed on a column of silica gel (100 Gm., Davison, commercial grade) prepared in a slurry with Skellysolve B. Elution with 20% ethyl acetate in Skellysolve B gave, after recrystallization from dilute ethanol, 0.8 Gm. of colorless needles m.p. 143–144°, which separated into two spots on a chromatostrip. One other zone was eluted in trace amounts.

(+)-*Cis*-**ethylkhellactone (XI).**[6]—The material obtained above (0.8 Gm.) was rechromatographed on a similar column. Development with 10% ethyl acetate in Skellysolve B separated two zones. Elution of the lowest zone with the same solvent gave (+)-*cis*-ethylkhellactone. Repeated crystallizations from dilute ethanol and drying at 78° in vacuum overnight afforded fine colorless needles, m.p. 127–128°; $[\alpha]_D^{24}$ +136° (c 1.00, C_2H_5OH); ultraviolet spectrum: $\lambda_{min.}$ 243 (log ε 3.47), 253 (3.44), and 266 mμ (3.18); $\lambda_{max.}$ 218 (4.12) sh., 247 (3.51), 258 (3.48); 300 (3.93) sh., and 327 mμ (4.19); infrared spectrum (Nujol mull): 3521 (OH), 1716 (α-pyrone C=O), 1626 (α-pyrone ring C=C), 1608, 1490 (aromatic C=C), 1567 (aromatic ring with conjugated C=C), 1342, 1104 (O—H deformation and C—O stretching), 1121 (Ar—O—CR_3), 1057 (ethyl ether), 889 [$(CH_3)_2C$—O] and 838 cm.$^{-1}$ (1,2,3,4-aromatic substitution).

Anal.—Calcd. for $C_{16}H_{18}O_5$: C, 66.19; H, 6.25; mol. wt., 290.30. Found: C, 65.75; H, 6.14; mol. wt. (Rast), 285.

(−)-*Trans*-**ethylkhellactone (IX).**—Further elution of the above column afforded (−)-*trans*-ethylkhellactone. Recrystallization from dilute methanol and drying at 100° in vacuum overnight gave fine colorless needles, m.p. 161–162°; $[\alpha]_D^{23}$ −64° (c 0.5, C_2H_5OH) [lit. m.p. 161–162°, $[\alpha]_D$ −59° (c 0.9, C_2H_5OH) (2)]; ultraviolet spectrum: $\lambda_{min.}$ 266 mμ (log ε 3.18); $\lambda_{max.}$ 327 mμ (4.19); infrared spectrum (Nujol mull) was similar to that of the (+)-*cis*-isomer except for the following major differences: 3636 (OH), 1323 and 1082 cm.$^{-1}$ (OH deformation and C—O stretching.)

The melting point was not depressed on admixture with an authentic sample of (−)-*trans*-ethylkhellactone.[7] Its ultraviolet and infrared spectra were identical to those of the authentic sample. This same compound was obtained by refluxing suksdorfin with 0.5 *N* ethanolic sodium hydroxide for 30 minutes, followed by the same work-up, and purified by repeated crystallizations from dilute methanol.

Anal.—Calcd. for $C_{16}H_{18}O_5$: C, 66.19; H, 6.25; mol. wt., 290.30. Found: C, 66.06; H, 6.40; mol. wt. (Rast), 286.

Isolation and Identification of Acids.—The rapid, horizontal paper chromatographic method of Roberts and Bucek (12) was used to obtain a preliminary characterization of the acids released by hydrolysis. Samples of pteryxin and suksdorfin (25 mg.) were refluxed with 1 *N* sodium hydroxide in 75% ethanol for 30 minutes. The solutions were acidified with concentrated sulfuric acid, adjusted to pH 8 with 33% aqueous ethylamine, and spotted on the paper. After development with a butanol-water solvent system, the acid spots were located by spraying the paper with 0.2% ethanolic chlorophenol red. Pteryxin gave two spots which corresponded quite closely with control samples of acetic and angelic acids, and suksdorfin, with acetic and isovaleric acids.

Pteryxin (1.0 Gm.) was refluxed with 1 *N* ethanolic potassium hydroxide (20 ml.) for 1 hour. The reaction mixture was diluted with water (50 ml.), freed of ethanol under reduced pressure, adjusted to pH 8 with concentrated sulfuric acid, and extracted with ether. The ether extract was evaporated to dryness. The *p*-phenylphenacyl esters were prepared from the residue and chromatographed on a silicic acid-Celite (3:1) column in the manner described by Klohs, *et al.* (13). Elution of the column with 1:1 benzene-petroleum ether (60–110°) yielded in the first fraction unreacted bromide, m.p. 124°. The second fraction afforded colorless crystals which, after recrystallization from ethanol, gave *p*-phenylphenacyl angelate as shiny platelets, m.p. 87–88° [lit. m.p. 90–91° (14)]. The melting point was not depressed on admixture with an authentic sample of *p*-phenylphenacyl angelate.[8]

Anal.—Calcd. for $C_{19}H_{18}O_3$: C, 77.53; H, 6.16. Found: C, 77.54; H, 6.16.

Further elution with 2:1 benzene-petroleum ether (60–110°) yielded colorless needles. Recrystallization from ethanol and drying at 100° in vacuum gave *p*-phenylphenacyl acetate as fine colorless needles, m.p. 109–110° [lit. m.p. 110–111° (13)]. The melting point was not depressed on admixture with the same derivative prepared from acetic acid.

Anal.—Calcd. for $C_{16}H_{14}O_3$: C, 75.58; H, 5.55. Found: C, 75.37; H, 5.73.

The *p*-phenylphenacyl esters of the acids obtained from suksdorfin in the same manner afforded *p*-phenylphenacyl acetate, m.p. 109–110°, no depression on admixture with the authentic sample, and *p*-phenylphenacyl isovalerate as shiny platelets, m.p. 78.5–79° [lit. m.p. 78° (15)]. The melting point was not depressed on admixture with the same derivative prepared from isovaleric acid.

Anal.—Calcd. for $C_{19}H_{20}O_3$: C, 77.01; H, 6.80. Found: C, 76.94; H, 6.94.

(−)-*Cis*-**acetylethylkhellactone (VII).**—A mixture of (+)-*cis*-ethylkhellactone (230 mg.), acetic anhydride (2 ml.), and dry pyridine (2 ml.) was heated on a steam bath for 1 hour and poured into water

[6] The compounds described in this paper have been named according to the scheme followed by Schroeder, *et al.* (4).

[7] Graciously supplied by Dr. Eric Smith, S. B. Penick and Co., New York, N. Y.

[8] Graciously supplied by Dr. S. W. Pelletier, the Rockefeller Institute for Medical Research, New York, N. Y.

(40 ml.). Upon standing overnight fine needles formed and were filtered from the liquor, leaving a clear viscous oil which had separated on the bottom of the beaker. The needles were recrystallized twice from dilute methanol and dried at 78° in vacuum to give (−)-*cis*-acetylethylkhellactone as colorless prisms, m.p. 122–123°; $[\alpha]_D^{25}$ −15° (c 0.527, C_2H_5OH); ultraviolet spectrum: $\lambda_{min.}$ 263 mμ (log ε 3.20); $\lambda_{max.}$ 325 mμ (4.18); infrared spectrum (Nujol mull): 1247 and 1043 cm.$^{-1}$ (acetate C—O).

Anal.—Calcd. for $C_{18}H_{20}O_6$: C, 65.05; H, 6.07. Found: C, 64.67; H, 6.26.

(−)-*Trans*-**acetylethylkhellactone (V).**—The residual oil obtained above crystallized upon rubbing with methanol. Three recrystallizations from dilute methanol followed by drying at 78° in vacuum gave (−)-*trans*-acetylethylkhellactone as colorless needles, m.p. 168–168.5°; $[\alpha]_D^{24}$ −39° (c 1.77, C_2H_5OH) [lit. m.p. 170–172°, $[\alpha]_D$ −40.6° (c 0.6, C_2H_5OH) (2)]; ultraviolet spectrum: $\lambda_{min.}$ 264 mμ (log ε 3.26); $\lambda_{max.}$ 325 mμ (4.20); infrared spectrum (Nujol mull): 1245 and 1042 cm.$^{-1}$ (acetate C—O).

Anal.—Calcd. for $C_{18}H_{20}O_6$: C, 65.05; H, 6.07. Found: C, 64.90; H, 6.14.

This acetate was obtained from (−)-*trans*-ethylkhellactone in the same manner.

Methanolic Alkaline Hydrolysis.—Crude pteryxin (15 Gm.) dissolved in methanol (60 ml.) was mixed with 1 *N* methanolic sodium hydroxide (60 ml.) and refluxed 90 minutes. The brown mixture was diluted with water (300 ml.) and the methanol removed under reduced pressure. The aqueous solution was extracted with three 100-ml. portions of methylene chloride, which were combined and evaporated to dryness. The light brown residue (4.0 Gm.) was recrystallized from methanol, then ether-Skellysolve B as colorless needles (2.5 Gm.), which separated into two spots on a chromatostrip developed with 50% ethyl acetate in Skellysolve B; m.p. 159–160°; $[\alpha]_D^{24}$ +37° (c 1.00, CH_3OH), corresponding to a mixture of 40% (+)-*cis*- and 60% (−)-*trans*-methylkhellactone.[9]

(+)-*Cis*-**methylkhellactone (XII).**—The above mixture (731 mg.) was chromatographed on neutral alumina (25 Gm., Woelm, activity III, prepared according to Brockmann). Elution with benzene gave (+)-*cis*-methylkhellactone (295 mg.), m.p. 126–127°; $[\alpha]_D^{23}$ +88° (c 0.752, C_2H_5OH) [lit. m.p. 127°, $[\alpha]_D^{22}$ +97° (c 0.628, CH_3OH) (4)]; ultraviolet spectrum: $\lambda_{min.}$ 266 mμ (log ε 3.18); $\lambda_{max.}$ 329 mμ (4.21); infrared spectrum (Nujol mull): 3521 (OH), 1351 and 1111 cm.$^{-1}$ (OH deformation and C—O stretching).

Anal.—Calcd. for $C_{15}H_{16}O_5$: C, 65.19; H, 5.84. Found: C, 65.20; H, 6.01.

(−)-*Trans*-**methylkhellactone (X).**—Further elution of the above column with 1:4 ether-benzene gave (−)-*trans*-methylkhellactone (313 mg.) which was recrystallized from ether-petroleum ether (30–60°) as colorless needles, m.p. 160–161°; $[\alpha]_D^{25}$ − 8° (c 0.793, C_2H_5OH) [lit. m.p. 161.5–162.5°, $[\alpha]_D^{24}$ −3° (c 1.374, CH_3OH) (4)]; ultraviolet spectrum: $\lambda_{min.}$ 265 mμ (log ε 3.32); $\lambda_{max.}$ 328 mμ (4.21); infrared spectrum (Nujol mull): 3571 (OH), 1328 and 1101 cm.$^{-1}$ (OH deformation and C—O stretching).

Anal.—Calcd. for $C_{15}H_{16}O_5$: C, 65.19; H, 5.84. Found: C, 65.44; H, 6.03.

On acetylation, the methyl ether mixture (300 mg.) gave, after recrystallization from dilute methanol and drying at 100° in vacuum, (+)-*trans*-acetylmethylkhellactone (VI) as colorless needles (200 mg.), m.p. 148–149° [lit. m.p. 152.5° (4)]; ultraviolet spectrum: $\lambda_{min.}$ 263 mμ (log ε 3.14); $\lambda_{max.}$ 325 mμ (4.18).

Anal.—Calcd. for $C_{17}H_{18}O_6$: C, 64.14; H, 5.70. Found: C, 64.14; H, 5.68.

Quantitative Hydrolytic Experiments.—Samples of recrystallized pteryxin (61.8 mg.), suksdorfin (61.4 mg.), visnadin[10] (51.5 mg.), and (+)-*trans*-acetylmethylkhellactone were each dissolved in 0.25 *N* ethanolic sodium hydroxide (10.0 ml.) at room temperature. Aliquots (1.0 ml.) taken at various intervals were diluted with carbon dioxide-free water (15 ml.) and titrated with 0.0243 *N* sulfuric acid to pH 7 on a Beckman Zeromatic pH meter. A blank determination of the alkali solution was made at the same time. The results expressed as mole equivalents plotted against time in minutes are shown in Fig. 4.

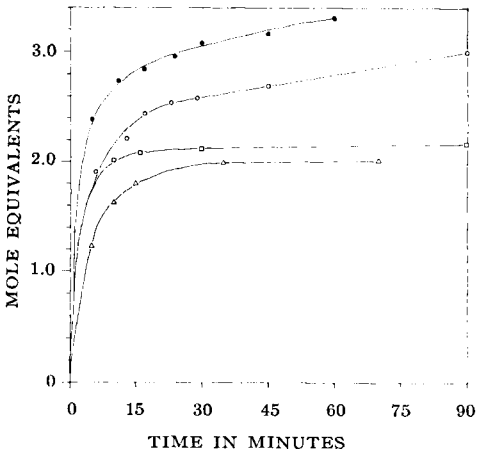


Fig. 4.—Hydrolysis rates in 0.25 *N* ethanolic sodium hydroxide at room temperature. ●, suksdorfin; ○, pteryxin; □, visnadin; △, (+)-*trans*-acetylmethylkhellactone.

Controlled Hydrolysis of Pteryxin.—Recrystallized pteryxin (1.0 Gm., 2.6 mmole) was dissolved in 0.25 *N* ethanolic sodium hydroxide (25.0 ml.) at room temperature and the rotation taken every 30 seconds. The rotation decreased rapidly for 8 minutes, remained constant for the next 4 minutes, and then started to decrease. An aliquot (1.0 ml.) was taken quickly and added to water (15 ml.) as the remaining solution was simultaneously added to an equivalent amount of sulfuric acid (6 meq.) in water (250 ml.). The reaction period was 13 minutes. The diluted aliquot was titrated im-

[9] It should be pointed out that Smith, *et al.* (2), obtained from samidin a methyl ether which melted at 158–159° and gave a rotation of $[\alpha]_D$ +14°, which is a mixture of approximately 83% *trans*- and 17% *cis*-isomers. Schroeder, *et al.* (4), have drawn attention to the fact that in no case did these workers report isomeric pairs, even though the isomers had been previously characterized in a preliminary report by the Swiss workers (16).

[10] Graciously supplied by Dr. Hamed Abu-Shady, Pharm. Chem. Department, Cairo University, Cairo, Egypt.

mediately with 0.0243 *N* sulfuric acid; a blank of the alkali solution was titrated at the same time. The alkali consumed was 1.75 mole equivalents. The aqueous acid solution of the reaction mixture was extracted with five 25-ml. portions of 3:1 ether-methylene chloride. The combined ethereal extract was washed with 10% sodium bicarbonate solution followed by saturated sodium chloride solution. The washed extract was dried over anhydrous sodium sulfate and evaporated to dryness. The residue was dried to constant weight in vacuum (680 mg.) and chromatographed on a column of neutral alumina (15 Gm., Woelm, Brockmann activity III). Elution with benzene yielded a crystalline residue from the first fraction (20 ml.). Recrystallization from methanol and drying at 78° in vacuum overnight afforded (−)-*trans*-acetylethylkhellactone (V), m.p. 167–168°. The melting point was not depressed on admixture with the acetate prepared from (−)-*trans*-ethylkhellactone. Their infrared spectra were superimposable.

Anal.—Calcd. for $C_{18}H_{20}O_6$: C, 65.05; H, 6.07. Found: C, 64.90; H, 6.15.

Continued elution with benzene, after removal of a dark zone, yielded a second crystalline fraction which was recrystallized from dilute methanol and dried at 78° in vacuum to give (+)-*cis*-ethylkhellactone (XI) as very fine colorless needles, m.p. 126–127°. The melting point was not depressed on admixture with a sample of (+)-*cis*-ethylkhellactone obtained previously from pteryxin. Their infrared spectra were identical.

Anal.—Calcd. for $C_{16}H_{18}O_5$: C, 66.19; H, 6.25. Found: C, 66.17; H, 6.36.

Further elution afforded only trace amounts of material.

Controlled Hydrolysis of Suksdorfin.—Suksdorfin (1.00 Gm., 2.58 mmole) was hydrolyzed in the same manner as above. The reaction period was 5 minutes with 1.65 mole equivalents of alkali being consumed. The reaction mixture was worked-up and the residue chromatographed as above. (−)-*Trans*-acetylethylkhellactone, m.p. 169–170°, and (+)-*cis*-ethylkhellactone, m.p. 127–128°, were obtained. Mixed melting points with samples obtained previously showed no depression and their infrared spectra were identical. Further elution of the column afforded only trace amounts of material.

3′-Keto-3′,4′-dihydroseselin (XIII).—Ethylkhellactone (2.18 Gm.) was treated with *p*-toluenesulfonic acid in boiling benzene in the same manner described by Schroeder, *et al.* (4). Recrystallization from 1:1 acetone-Skellysolve B gave 1.08 Gm. (59%) of light yellow prisms, m.p. 157–157.5° [lit. m.p. 156.5–157.5° (4)]; ultraviolet spectrum: $\lambda_{min.}$ 263 mμ (log ε 3.40); $\lambda_{max.}$ 321 mμ (4.10); infrared spectrum (Nujol mull): 1757 cm.$^{-1}$ (α- and β-pyrone C=O).

Anal.—Calcd. for $C_{14}H_{12}O_4$: C, 68.86; H, 4.95. Found: C, 68.65; H, 4.99.

(±)-3′-Hydroxy-3′,4′-dihydroseselin (XIV).—To a stirred solution of 3′-keto-3′,4′-dihydroseselin (488 mg.) in methanol (40 ml.), cooled to −10° in an ice-salt mixture, was added dropwise an ice-cold solution of sodium borohydride (530 mg.) in methanol (10 ml.) over a 15-minute period. The mixture was stirred an additional 45 minutes at room temperature. The yellow solution was made slightly acidic with 1 *N* hydrochloric acid and concentrated to 10 ml. Upon standing overnight fine red needles formed which were filtered off and air-dried to yield 422 mg. (86%) of product. Repeated crystallizations from dilute ethanol gave pale pink needles, m.p. 165–166°; infrared spectrum (Nujol mull): 3636 (free OH), 1720 (C=O), 1295 and 1090 cm.$^{-1}$ (O—H deformation and C—O stretching).

Anal.—Calcd. for $C_{14}H_{14}O_4$: C, 68.28; H, 5.73. Found: C, 68.00; H, 5.60.

Acetylation of 200 mg. of the alcohol XIV with acetic anhydride and pyridine gave 220 mg. (94%) of acetate. Recrystallization from dilute ethanol gave fine colorless needles, m.p. 172–173°; infrared spectrum (Nujol mull): 1752–1745 (α-pyrone and acetate C=O) and 1242 cm.$^{-1}$ (acetate C—O).

Anal.—Calcd. for $C_{16}H_{16}O_5$: C, 66.65; H, 5.59. Found: C, 66.50; H, 5.70.

The nuclear magnetic resonance (n.m.r.) spectra of the alcohol XIV and its acetate is in complete agreement with the assigned structures.[11]

DISCUSSION

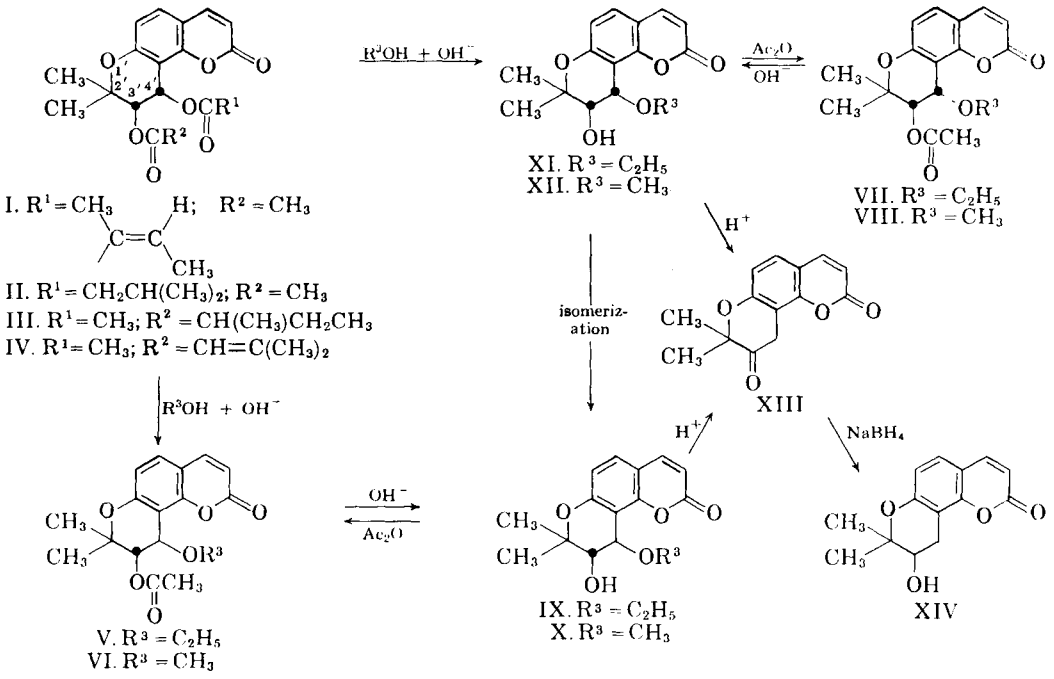
Pteryxin (I) was isolated in 2% yield from dried roots of *P. terebinthina* and suksdorfin (II) in 1.2% yield from dried fruits of *L. Suksdorfii*. Chromatostrips were utilized in finding a satisfactory solvent system to purify these substances. Results obtained on the chromatostrips were readily adaptable to column chromatography and were a definite labor-saving device.

Ultraviolet spectra of these substances and their derivatives had absorption maxima at 322–329 mμ and minima at 263–266 mμ, which are in agreement with their assigned coumarin structures (2).

Upon hydrolysis with ethanolic alkali, pteryxin (I) and suksdorfin (II) both yielded the same isomeric alcohols, (−)-*trans*-ethylkhellactone (IX), which was shown to be identical to the hydrolysate obtained from samidin by Smith, *et al.* (2), and (+)-*cis*-ethylkhellactone (XI). In methanol, the corresponding methyl ethers (X and XII) were obtained. Absolute configurational formulas for the methylkhellactones were established by Schroeder, *et al.* (4). These ethers arise by means of a solvolysis of the 4′-position in the parent compounds, a finding substantiated by both Smith (2) and Schroeder (4). The 4′-position constitutes a benzyl ester, certain ones of which are known to undergo solvolysis with O-alkyl cleavage under acidic or basic conditions (17).

(+)-*Cis*-ethylkhellactone (XI) and its acetate (VII) have not been reported previously. We assigned absolute configurational structures to them on the basis of similarities to the established methyl ethers. The *cis* relationship of the adjacent hydroxyl and ethoxyl groups is indicated by the hydrogen bonding of the hydroxyl group evidenced in its infrared spectrum (3521 cm.$^{-1}$) as compared to the free hydroxyl shown by the *trans*-isomer (3636 cm.$^{-1}$). Additional evidence for hydrogen bonding in the *cis*-isomer is the location of its secondary hydroxyl OH deformation and C—O stretching absorption at 1342 and 1104 cm.$^{-1}$, whereas these peaks are at 1323 and 1082 cm.$^{-1}$ for the *trans*-

[11] The authors thank Mr. George Slomp, The Upjohn Co. Kalamazoo, Mich., for the determination and interpretation of the n.m.r. spectra.



isomer. Bonded hydroxyls are known to absorb at lower wavelengths (18). The same is true of the methyl ethers.

Ethylkhellactone (IX + XI) was dehydrated to form the ketone XIII, which was reduced with sodium borohydride to the alcohol XIV. The structures for alcohol XIV and its acetate were confirmed by nuclear magnetic resonance spectra.

Hydrolysis of pteryxin yielded angelic and acetic acids, and suksdorfin yielded isovaleric and acetic acids. These were characterized by paper chromatography and through their *p*-phenylphenacyl ester derivatives.

In a titration experiment measuring the rate of alkali consumption of visnadin (III) and samidin (IV) during room temperature hydrolysis in ethanolic alkali, Smith, *et al.* (2), reported these coumarins to consume only approximately two equivalents of alkali after 90 minutes. The third equivalent was consumed after refluxing. Also, they were able to isolate a room temperature hydrolysate which contained a five carbon acid moiety at the 3′-position. In a similar experiment, pteryxin (I) consumed all three equivalents at room temperature in 90 minutes, and suksdorfin (II) in 30 minutes.

Esters of α,β-unsaturated acids and higher saturated fatty acids hydrolyze in acid or base at a significantly slower rate than acetates (19). Clearly, the α,β-unsaturated angeloyl group in pteryxin and the isovaleryl group in suksdorfin are not on the 3′-oxygen, as is the case in visnadin and samidin, but are on the 4′-oxygen, where the solvolysis rate is not affected by the nature of the acid substituent. It follows therefore, that the acetoxy group is at the 3′-position and, inasmuch as it offers less resistance to hydrolysis, is cleaved at room temperature in a relatively short period of time.

If the assignment of the acetoxy group to the 3′-position is correct, then the acetate of the intermediate ether (V or VII) should hydrolyze at a similar rate. Such was the case. Hydrolysis of (+)-*trans*-acetylmethylkhellactone (VI) was complete in 35 minutes.

By quenching the hydrolysis of the parent coumarins after 5 to 13 minutes, a mixture of (+)-*trans*-ethylkhellactone acetate (V) and (+)-*cis*-ethylkhellactone (XI) was obtained. Isolation of the intermediate acetate (V), which was identical to that prepared from the alcohol, conclusively proves pteryxin and suksdorfin to have structures I and II, respectively.

Migration of the acyl or acyloxy group from the 4′-position, to the 3′-position, which were arbitrarily designated as *trans*, was discussed by Smith, *et al.* (2), and the possibility eliminated. Establishment of their *cis* configuration makes a migration even more remote.

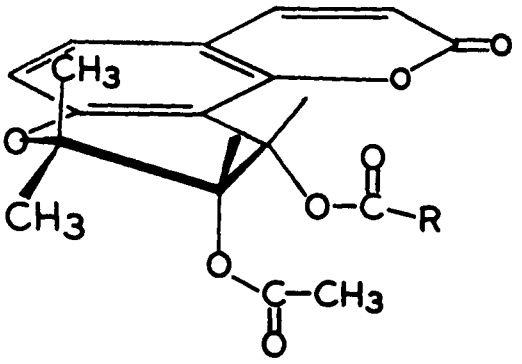
The hydrolytic studies indicated that the 3′-acetyl group was being hydrolyzed concomitantly, although at a slower rate, with the loss of the angeloyloxy group. As indicated above, the partial hydrolysis product was the *trans*-acetate (V) and the initial completely hydrolyzed one the *cis*-alcohol XI. If the reaction was allowed to proceed for an additional 15 minutes, the major product was the *trans*-alcohol IX, which arises by hydrolysis of the acetate V and by isomerization of the less stable *cis*-alcohol XI.

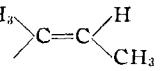
The hydrolytic results described above may be rationalized on the basis that the system is undergoing two different competitive processes, the one being kinetically controlled and relatively fast and the other being a slower equilibrium process. The former leads to the less stable *cis*-isomer and the latter predominantly to the more stable *trans*-isomer. It may be considered that the process, as postulated by Smith, *et al.* (2), involves first a fast S_N1 type expulsion of the 4′-acyloxy group initiated by the neighboring phenolic group. This leads to an enone intermediate which stabilizes the resulting carbonium ion. A study of structural models of the species involved leads us to suggest that the results of the

hydrolytic studies may be explained plausibly if one considers that the 3′-acetoxy group is undergoing continuous hydrolysis from the moment it is subjected to alkaline conditions. Thus, at all times, we can postulate two species of carbonium ion present, one with an acetoxy group and one with only a hydroxyl group at the 3′-carbon atom. The arrangement of groups on this carbon atom offers the strong possibility of a neighboring group type participation between the quasi-axially oriented acetoxy carbonyl and the carbonium ion center. Stabilization of the acetoxy group in this manner would reasonably promote ethoxide ion addition on the less hindered *trans*-side and lead to the *trans*-acetate V. On the other hand, the alcoholic species would no longer have this stabilizing influence and, therefore, both *cis*- and *trans*-addition can be envisioned. In this respect, the *cis*-addition, favored by a serious axial methyl (at the 2′-position) interference with *trans*-addition, is suggested as being quite rapid kinetically and would account for the isolation of the *cis*-alcohol XI when the reaction is quenched before equilibrium has been achieved. As the process proceeds, however, equilibration finally results in a preponderance of the *trans*-alcohol IX over the *cis*-alcohol, the former being obtained by hydrolysis of V as well as by isomerization of the less stable XI under alkaline conditions.

Pteryxin (I) and suksdorfin (II) have the same configuration as visnadin (III) and samidin (IV) at the 3′-position, as evidenced by the formation of the same hydrolytic products. The absolute configurational structures for the latter pair of compounds were established by Schroeder, *et al.* (4), on the basis of their (+)-rotation and the similarities of their infrared spectra in the 9 and 10 μ region to synthetic (±)-*cis*-diacetylkhellactone. Also, these infrared spectra differed significantly from those of synthetic (±)-*trans*-samidin and (±)-*trans*-diacetylkhellactone. Since pteryxin (I) and suksdorfin (II) possess these features too, they have the same configuration as visnadin (III) and samidin (IV) at the 4′-position, and are, therefore, established as (+)-*cis*-pteryxin and (+)-*cis*-suksdorfin.

We propose the following absolute conformational structures for (+)-*cis*-pteryxin (XV) and (+)-*cis*-suksdorfin (XVI) with the 3′-acetoxy and 4′-acyloxy groups in axial and quasi-equatorial positions, respectively. The arrangement of these two groups in molecular models is distorted from the normal axial and equatorial positions that would be encountered if the ring system were a cyclohexane type rather than the fused dihydro-pyrano system that actually exists. This, no doubt, is due to the different bond lengths and angles of the ether oxygen.



XV: R = 

XVI: R = $CH_2CH(CH_3)_2$

The mechanisms involved in the hydrolysis of these coumarin esters and additional stereochemical proofs are presently under further investigation.

REFERENCES

(1) Huttrer, C. P., and Dale, E., *Chem. Revs.*, **48**, 543 (1951).
(2) Smith, E., *et al.*, *J. Am. Chem. Soc.*, **79**, 3534(1957).
(3) "Provismine," unpublished bulletin, Memphis Chemical Co., 30, Wabour El-Miyah, Zeitoun, Egypt.
(4) Schroeder, H. D., *et al.*, *Chem. Ber.*, **92**, 2338(1959).
(5) Call, T. G., Ph.D. thesis, University of Minnesota, 1956.
(6) Call, T. G., and Fischer, E. B., *Northwest Sci.*, **32**, 96(1958).
(7) Pettinato, F. A., M.S. thesis, Montana State University, 1954.
(8) Call, T. G., and Green, J., *Proc. Montana Acad. Sci.*, **16**, 49(1956).
(9) Pettinato, F. A., Fischer, L., and Hall, N. A., THIS JOURNAL, **48**, 423(1959).
(10) *Ibid.*, **49**, 45(1960).
(11) Stanley, W. L., Vannier, S. H., and Gentile, B., *J. Assoc. Offic. Agr. Chemists*, **282**, 582(1957).
(12) Roberts, H. R., and Bucek, W., *Anal. Chem.*, **29**, 1447(1957).
(13) Klohs, M. W., *et al.*, *J. Am. Chem. Soc.*, **74**, 5109 (1952).
(14) Buckles, R. E., Mock, G. V., and Locatell, L., *Chem. Revs.*, **55**, 659(1955).
(15) Cheronis, N. D., and Entrikin, J. B., "Semimicro Qualitative Organic Analysis," 2nd ed., Interscience Publishers, New York, N. Y., 1957, p. 553.
(16) Bencze, W., Halpern, O., and Schmid, H., *Experientia*, **12**, 137(1956).
(17) Davies, A. G., and Kenyon, J., *Quart. Revs.*, **9**, 203(1955).
(18) Bellamy, L. J., "The Infra-red Spectra of Complex Molecules," 2nd ed., John Wiley & Sons, New York, N. Y., 1958, p. 108.
(19) Newman, M. S., "Steric Effects in Organic Chemistry," John Wiley & Sons, New York, N. Y., 1956, p. 201.