The crude salt was stirred into a mixture of water, ether, and a slight excess of $NaHCO_3$ to convert it to the aldehyde. After 5 min the layers were separated, and the organic solid was isolated in the usual way. Direct conversion of 1 to 4-bromo-2-pyrrolecarboxaldehyde resulted in yields of 92% (bromination at 28°, 0.5 hr) and 89% (0°, 16 hr), mp 122.5–124.5° (C_6H_6) (lit.[6] mp 123–124°).

Minor amounts of the 5 isomer were identified by glc comparison with the bromination product from 2-pyrrolecarboxaldehyde prepared as described by Anderson and Lee.[6]

4-Acetyl-2-pyrrolecarboxaldehyde.—Acetyl chloride (0.54 ml) was injected into a violet solution of 1.25 g of 1 and 1.47 g of $AlCl_3$ in 25 ml of CH_2ClCH_2Cl at 0°. The resulting brown mixture was kept at 0° for 16 hr. The mixture was poured over crushed ice, and an aqueous solution of 2 g of NaOH was added. After the mixture had been stirred for 10 min, it was acidified (HCl) and extracted continuously with Et_2O (12 hr). The extract was dried (Na_2SO_4) and concentrated to give 0.54 g (77%), mp 139–142° (C_6H_6) (lit.[7b] 136–137°).

4-Acetyl-2-pyrrolecarboxylic Acid.—Silver nitrate (0.94 g) was dissolved in 95 ml of H_2O and added to 190 ml of 1 *N* NaOH. A solution of 0.51 g of 4-acetyl-2-pyrrolecarboxaldehyde in 38 ml of ethanol was added thereto and the resulting mixture was stirred for 0.5 hr. The mixture was filtered, acidified with HCl, and extracted continuously with ether for 6 hr. The extract was dried ($MgSO_4$) and concentrated to give 0.49 g of the acid, mp ~220° dec (lit.[7b] mp 221.5–223° dec).

Registry No.—1, 27521-94-4; 2, 27521-95-5.

Acknowledgment.—The author wishes to express his gratitude to M. Jacobson and N. Wakabayashi of this division for generously reading and commenting upon this manuscript.

Sterol Metabolism. XIV. Cholesterol 24-Hydroperoxide[1]

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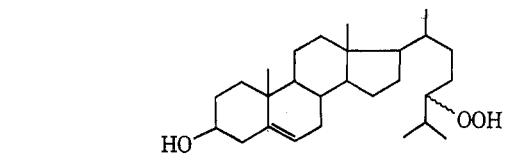
Received July 21, 1970

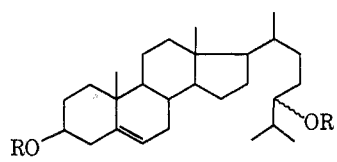
We have isolated from air-aged cholesterol the tertiary hydroperoxides, 3β-hydroxycholest-5-ene 20α-hydroperoxide and 3β-hydroxycholest-5-ene 25-hydroperoxide.[2] A third cholesterol hydroperoxide X_1, previously shown not to be the 17α-hydroperoxide, is identified herein as an epimeric mixture of the 3β-hydroxycholest-5-ene 24-hydroperoxides (I).

Sodium borohydride reduction of the hydroperoxide X_1 gave a mixture of epimeric diols from which one epimer was recovered by crystallization and identified as cholest-5-ene-3β,24ξ²-diol (IIa)[3,4] and from which both cholest-5-ene-3β,24-diol epimers were recovered and identified as their dibenzoates IIb. The 3β,24-diol structure for the cholest-5-ene-3β-24ξ²-diol epimer was suggested by its mass spectrum, which resembled in detail the mass spectra (above *m/e* 200) of the epimeric cholest-5-ene-3β-23-diols.[7] The 3β,24ξ-diols IIa (and their dibenzoates IIb) were distinguished from the known 17α-, 20α-, 22*R*-, 22*S*-, 23*R*-, 23*S*-, 25-, and 25*R*-26-monohydroxylated derivatives of cholesterol but were chromatographically similar to the 3β,24-diol cerebrosterol isolated from human and equine brain.[5] Comparison of the 3β,24ξ²-diol IIa and of the epimeric 3β,24-diol dibenzoates IIb obtained from the hydroperoxide X_1 with authentic sterols established their identity and thereby the identity of the hydroperoxide X_1 as an epimeric mixture of 3β-hydroxycholest-5-ene 24-hydroperoxides (I).

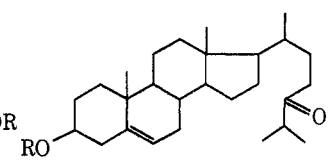
In distinction to the readily acetylated 20α- and 25-hydroperoxides of cholesterol,[2] the 24-hydroperoxides I decomposed on attempted acetylation with acetic anhydride–pyridine. Only 3β-acetoxycholest-5-en-24 one (IIIb) could be identified among the products formed.



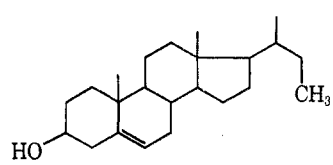
I



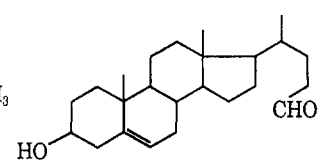
IIa, R = H
IIb, R = Bz



IIIa, R = H
IIIb, R = Ac



IV



V

The instability of the 24-hydroperoxides I to thermal and electron impact degradation was similar to that of the 20α- and 25-hydroperoxides. The three major products previously recognized[8] were identified by their chromatographic and spectral properties as 24-norchol-5-en-3β-ol (IV), 3β-hydroxychol-5-en-24-al (V), and 3β-hydroxycholest-5-en-24-one (IIa). The structure of the alcohol IV as 24-norchol-5-en-3β-ol rests on a consideration of the short gas chromatographic retention times on both 3% QF-1 and 3% SE-30 phases and the relatively high thin layer chromatographic mobility, which data imply a sterol of diminished carbon content. A magenta color with 50%

(1) Paper XIII of the series: J. E. van Lier and L. L. Smith, *J. Chromatogr.*, **49**, 555 (1970). Supported by funds from the U. S. Public Health Service (Grants NS-08106, HE-10160, and AM-13520) and from the Medical Research Council of Canada (Grant MA-4051) and the Conseil de la Recherche Médicale du Québec.

(2) J. E. van Lier and L. L. Smith, *J. Org. Chem.*, **35**, 2627 (1970).

(3) The original nomenclature for the 3β,24-diols IIa of Ercoli and de Ruggieri[4] is retained: cholest-5-ene-3β,24ξ¹-diol for the epimer named cerebrosterol occurring in human and equine brain,[5] cholest-5-ene-3β,24ξ²-diol for the epimer not found in nature. An absolute stereochemistry as the 3β,24β$_F$ (24*S*)-diol previously assigned[6] the 3β,24ξ¹-diol IIa has been questioned.[7]

(4) (a) A. Ercoli and P. de Ruggieri, *Gazz. Chim. Ital.*, **83**, 720 (1953); (b) A. Ercoli and P. de Ruggieri, *J. Amer. Chem. Soc.*, **75**, 3284 (1953).

(5) (a) A. Ercoli, S. Di Frisco, and P. de Ruggieri, *Boll. Soc. Ital. Biol. Sper.*, **29**, 494 (1953); (b) S. Di Frisco, P. de Ruggieri, and A. Ercoli, *ibid.*, **29**, 1351 (1953); (c) A. Ercoli, S. Di Frisco, and P. de Ruggieri, *Gazz. Chim. Ital.*, **83**, 78 (1953); (d) L. F. Fieser, W.-Y. Huang, and B. K. Bhattacharyya, *J. Org. Chem.*, **22**, 1380 (1957).

(6) W. Klyne and W. M. Stokes, *J. Chem. Soc.*, 1979 (1954).

(7) J. E. van Lier and L. L. Smith, *J. Pharm. Sci.*, **59**, 719 (1970).

(8) J. E. van Lier and L. L. Smith, *Steroids*, **15**, 485 (1970).

sulfuric acid and infrared absorption spectra support the Δ^5-3β-alcohol feature. The strong molecular ion at *m/e* 330 in the mass spectrum of IV together with the ion at *m/e* 273 (M − C_4H_9) representing loss of an unfunctionalized *sec*-butyl side chain complete the proof of structure. Notably no cholest-5-ene-3β,24-diols were formed thermally from the 24-hydroperoxides I.

The mass spectrum of the 24-hydroperoxides I showed a molecular ion at *m/e* 418 and an ion at *m/e* 402 representing loss of an atom of oxygen, which process also characterized the mass spectrum of 3β-hydroxycholest-5-ene 25-hydroperoxide.[8] Also significant were the molecular ions of the major thermal decomposition products including that of the 24-ketone III at *m/e* 400 (58%), the base peak ion at *m/e* 358 (100%) of the 24-aldehyde V, and an ion at *m/e* 330 (14%) corresponding to the alcohol IV.

The stereochemical composition of the 24-hydroperoxides I was checked on a sample isolated chromatographically without benefit of crystallizations. The epimeric 3β,24-diols obtained therefrom by borohydride reduction were shown by thin layer chromatographic analysis of their dibenzoates and by isolation to be a 1:2 mixture of the 3β,24ξ^1- and 3β,24ξ^2-diol epimers. On the assumption that the 24-hydroperoxides isolated were representative of those formed autoxidatively from cholesterol in the solid state, and that neither borohydride reduction nor benzoylation fractionated the epimers, the 3β,24-diol benzoate mixture recovered thus measured the composition of the 24-hydroperoxide I as a 1:2 mixture of epimers. The benzoylation and thin layer chromatographic analytical method had previously been carefully checked to show that known mixtures of the 3β,24ξ^1- and 3β,24ξ^2-diol dibenzoates in 1:1 to 1:8 ratios were correctly analyzed.[1]

Although complete stereospecificity is exhibited in the photosensitized formation in solution of steroid A- and B-ring allylic hydroperoxides,[9] competing radical attack in certain cases gave both possible epimers.[9c] In the absence of steric features of the cholesterol molecule which would provide a basis for selective approach of molecular oxygen in the formation of the 24-hydroperoxides I, we would predict formation of equal amounts of both 24-hydroperoxide epimers. The stereospecificity represented by the 1:2 ratio of epimers found implies a preference for autoxidative attack on one otherwise undistinguished face of the 24-carbon atom, which preference must derive from the orientation of the sterol side chain in the sterol crystal lattice.

Experimental Section[10]

3β-Hydroxycholest-5-ene 24-Hydroperoxides (I).—Air-aged cholesterol processed as previously described[2] gave a concentrate enriched in autoxidation products including the several hydroperoxides. By repeated alternate column chromatography on silica gel and on Sephadex LH-20, there was recovered 15 mg of I (yield 50 mg/kg of cholesterol), mp 160–165°, identical in spectral and chromatographic properties with the 24-hydroperoxide X_1 previously described.[2] The medium resolution mass spectrum of I has been published.[8] High resolution mass spectra included ions: *m/e* 418.3473 (calcd for $C_{27}H_{46}O_3$: 418.3446), 400.3370 (calcd for $C_{27}H_{44}O_2$: 400.3341), 358.2856 (calcd for $C_{24}H_{38}O_2$: 358.2872), and 330.2896 (calcd for $C_{23}H_{38}O$: 330.2923), etc.

Reduction of 3β-Hydroxycholest-5-en-24-one (IIIa).—A solution of 1.5 g of the 24-ketone IIIa[12] in methanol was reduced with an excess of sodium borohydride at 0°. After 10 min the solution was allowed to warm to room temperature, and after 12 hr the solution was treated with 0.1 *N* hydrochloric acid and the sterols were recovered by extraction with diethyl ether. The ether extract was washed with water, sodium bicarbonate solution, and brine, dried over anhydrous sodium sulfate, and evaporated under vacuum. The residue was chromatographed on a 60 × 2.5 cm column on Sephadex LH-20[11b] using methylene chloride. The fractions containing the epimeric 3β,24-diols were shown to be composed of 47% of the naturally occurring epimer cholest-5-ene-3β,24ξ^1diol, 53% of the unnatural epimer cholest-5-ene-3β,24ξ^2-diol by thin layer chromatography of the dibenzoates.[1] The mixed epimers were benzoylated in dry pyridine using benzoyl chloride, and the crude dibenzoates were resolved by thin layer chromatography on 20 × 40 cm chromatoplates 1- and 2-mm thick of silica gel HF_{254}, using benzene–hexane (1:1) as irrigating solvent, run for 15 hr in ascending fashion. The steryl dibenzoate zones were located under 254-nm ultraviolet light and eluted from the chromatoplate with diethyl ether, and the esters recrystallized from methanol, thus yielding the pure epimeric dibenzoates free from one another. The dibenzoates were saponified by refluxing in methanolic 5% sodium methoxide for 3 days. To the cooled solution diethyl ether was added, and the ether layer separated, washed with water three times, dried over anhydrous sodium sulfate, and evaporated under vacuum. The free sterol was recrystallized from hexane–diethyl ether.

Reduction of 3β-Hydroxycholest-5-ene 24-Hydroperoxides.—A sample of the epimeric 24-hydroperoxides I (25 mg) obtained from cholesterol air oxidation without crystallization was dissolved in methanol and reduced with an excess of sodium borohydride for 10 min at room temperature. The solution was treated with 0.1 *N* hydrochloric acid, and the sterols were isolated in exactly the same fashion as described for the reduction of the 24-ketone IIIa. The crude IIa preparation was benzoylated as described, and the crude dibenzoate was analyzed by thin layer chromatography[1] as a mixture of 35% 3β,24ξ^1-diol dibenzoate and 65% 3β,24ξ^2-diol dibenzoate. The crude dibenzoate mixture was chromatographed on a 20 × 40 cm chromatoplate 1-mm thick with benzene–hexane (1:1) for 15 hr, and the two bands of dibenzoate products were located under 254-nm ultraviolet light, the silica gel was excised, and the steryl esters were recovered by extraction with diethyl ether. Each ester was recrystallized from methanol.

Cholest-5-ene-3β,24ξ^1-diol 3β,24ξ^1-Dibenzoate (IIb). A. From the 24-Ketone IIIa.—IIb was obtained in 100-mg yield: mp 179–182° (lit. mp 179–181°,[4] 182–183°[5d]); λ_{max}^{MeOH} 228 nm (ϵ 24,400); ν_{max}^{KBr} 1710, 1280, 1110, 710 cm^{-1}.

B. From the 24-Hydroperoxides I.—IIb was obtained in 3-mg yield, mp 179–181°, identified by mixture melting point and infrared spectral comparisons and by chromatographic behavior with authentic cholest-5-ene-3β,24ξ^1-diol dibenzoate prepared under A above.

Cholest-5-ene-3β,24ξ^2-diol 3β,24ξ^2-Dibenzoate (II). A. From the 24-Ketone IIIa.—II was obtained in 90-mg yield: mp 149–150° (lit.[4] mp 141–142°); λ_{max}^{MeOH} 228 nm (ϵ 24,000); ν_{max}^{KBr} 1710, 1280, 1110, 710 cm^{-1}.

B. From the 24-Hydroperoxides I.—II was obtained in 7-mg yield, mp 138–141°, identified by mixture melting point and infrared spectral comparisons and by chromatographic behavior with authentic cholest-5-ene-3β,24ξ^2-diol dibenzoate prepared under A above.

The epimeric 3β,24-dibenzoates can be differentiated by their infrared absorption spectra, the fingerprint region having at least four distinguishing features: (1) a weak doublet at 668 and

(9) (a) A. Nickon and J. F. Bagli, *J. Amer. Chem. Soc.*, **81**, 6330 (1959); **83**, 1498 (1961); (b) A. Nickon and W. L. Mendelson, *Can. J. Chem.*, **43**, 1419 (1965); (c) A. Nickon, N. Schwartz, J. B. DiGiorgio, and D. A. Widdowson, *J. Org. Chem.*, **30**, 1711 (1965); (d) A. Nickon and W. L. Mendelson, *ibid.*, **30**, 2087 (1965).

(10) Experimental details of measurement of physical data, spectra, and chromatographic properties have been described previously in footnote 15 of ref 2. Preparative gas chromatography on 3% QF-1 columns was performed as previously described,[11a] as was preparative chromatography on Sephadex LH-20.[11b]

(11) (a) J. E. van Lier and L. L. Smith, *J. Chromatogr.*, **36**, 7 (1968); (b) *ibid.*, **41**, 37 (1969).

(12) B. Riegel and I. A. Kay, *J. Amer. Chem. Soc.*, **66**, 723 (1944).

680 cm^{-1}, the 668/680 ratio being approximately unity for the 24ξ^1 epimer, less than unity for the 24ξ^2 epimer; (2) absorption beginning below 900 cm^{-1} and a distinct band at 910 cm^{-1} for the 24ξ^1 epimer with no specific absorption at 900 cm^{-1} nor a band at 910 cm^{-1} for the 24ξ^2 epimer; (3) a complex multiplet of bands centered about 930 cm^{-1} for the 24ξ^1 epimer, around 945 cm^{-1} for the 24ξ^2 epimer; and (4) a well-formed doublet at 995 and 1005 cm^{-1}, the 995/1005 ratio being less than unity for the 24ξ^1 epimer, greater than unity for the 24ξ^2 epimer.

Cholest-5-ene-3β,24ξ^1-diol (cerebrosterol) (IIa) was obtained in 53-mg yield from its dibenzoate IIb prepared from IIIa: mp 175° (lit. mp 175–176°,[4] 170–171.5° to 173.5–175°[5d]); $\tilde{\nu}_{max}^{KBr}$ 3400, 1050, 672 cm^{-1}; R_c 0.75 (red-brown color with 50% sulfuric acid); t_R 2.36 (3% QF-1), 2.20 (3% SE-30); identified by direct comparison with authentic samples of cerebrosterol obtained from equine and human brain.

Cholest-5-ene-3β,24ξ^2-diol (IIa). A. From IIIa.—IIa was obtained in 51-mg yield from its dibenzoate IIb: mp 184–186° (lit.[4] mp 182–183°); $\tilde{\nu}_{max}^{KBr}$ 3400, 1050 cm^{-1} (no band at 672 cm^{-1}); R_c 0.75 (red-brown color with 50% sulfuric acid); t_R 2.36 (3% QF-1), 2.20 (3% SE-30).

B. From the 24-Hydroperoxide I.—IIa was obtained previously by borohydride reduction: mp 176–179°; R_c 0.77 (red-brown color with 50% sulfuric acid); identical in infrared and gas chromatogaphic properties with the 3β,24ξ^2-diol prepared under A above; mass spectrum *m/e* 402 (100), 384 (62), 369 (30), 351 (20), 317 (18), 291 (22), 273 (50), 255 (28), etc.

24-Norchol-5-en-3β-ol (IV).—Injection of 5–10 μg of I dissolved in 1–2 μl of chloroform–methanol (9:1) into the flash heater zone (250°) of a Hewlett-Packard F & M Model 402 gas chromatograph and collection of effluxing components in a glass capillary gave IV as the initially eluted component in 14% yield (unidentified component no. 1, 10% yield, in previous studies[8]). The collected sample was homogeneous by thin layer chromatography and by gas chromatography on both 3% QF-1 and 3% SE-30 phases and was characterized: R_c 1.00 (magenta color with 50% sulfuric acid); t_R 0.45 (3% QF-1), 0.38 (3% SE-30); $\tilde{\nu}_{max}^{KBr}$ 3400, 1620, 1060 cm^{-1}. The pure IV was transferred in diethyl ether to a quartz probe and inserted directly into the mass spectrometer to yield the molecular ion at *m/e* 330 (100), 315 (34, M − H_2O), 312 (46, M − CH_3), 297 (48, M − H_2O − CH_3), 273 (28, M − C_4H_9), 255 (37, M − C_4H_9 − H_2O).

3β-Hydroxychol-5-en-24-al (V).—The second component to efflux from the thermally decomposed sample of I was collected in a capillary in 46% yield (unidentified component no. 2, 50% yield previously[8]). The component was homogeneous by thin layer and gas chromatography: R_c 0.85 (magenta-red color with 50% sulfuric acid); t_R 2.40 (3% QF-1), 0.90 (3% SE-30); $\tilde{\nu}_{max}^{KBr}$ 3400, 1720, 1620, 1050 cm^{-1}, identical in these properties with an authentic sample; mass spectrum *m/e* 358 (100, molecular ion), 343 (23), 340 (56), 330 (10), 325 (33), 273 (40, M − C_4H_9O), 255 (20).

3β-Hydroxycholest-5-en-24-one (IIIa).—The third major thermal decomposition product of I was collected in a capillary in 27% yield (unidentified component no. 3, 40% yield previously[8]). The 24-ketone IIIa was homogeneous on thin layer and gas chromatographic analysis: R_c 0.95 (magenta-red color with 50% sulfuric acid); t_R 3.35 (3% QF-1), 1.68 (3% SE-30); $\tilde{\nu}_{max}^{KBr}$ 3400, 1700, 1620, 1060, 1020, 800 cm^{-1}; identical in these properties with an authentic sample; mass spectrum *m/e* 400 (87%, molecular ion), 385 (27), 382 (100), 367 (57), 315 (73), 314 (92), 299 (50), 297 (44), 296 (35), 289 (45), 281 (46), 273 (30, M − $C_8H_{15}O$), 271 (60), 255 (34).

3β-Acetoxycholest-5-en-24-one (IIIb).—Attempted acetylation of I with acetic anhydride–pyridine (1:2) overnight at room temperature in the usual manner resulted in total decomposition of the sterol hydroperoxide (negative peroxide tests). The major product was isolated in 35% yield by preparative gas chromatography, yielding pure IIIb homogeneous on thin layer and gas chromatograms: R_c 1.30 (magenta-red color with 50%, sulfuric acid); t_R 5.9 (3% QF-1); $\tilde{\nu}_{max}^{KBr}$ 1730, 1710, 1380, 1250, 1040 cm^{-1}; identical in these properties with an authentic sample.

Registry No.—I (24*R*), 27460-24-8; I (24*S*), 27460-25-9; IIa (24*R*), 27460-26-0; IIa (24*S*), 27460-27-1; IIb (24*R*), 27460-28-2; IIb (24*S*), 27460-29-3; IIIa, 17752-16-8; IIIb, 20981-59-3; IV, 27460-32-8; V, 27460-33-9.

Acknowledgment.—The authors thank Professor L. F. Fieser, Harvard University, for a reference sample of cerebrosterol from equine brain, and Dr. J. A. McCloskey, Baylor University School of Medicine, for mass spectra.

The Dieckmann Cyclization as a Route to *A*-Nor Steroids. Evidence Concerning Stereochemistry

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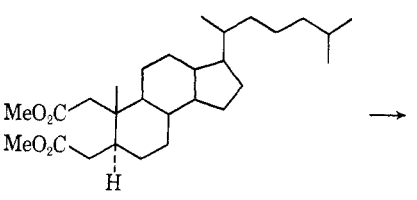
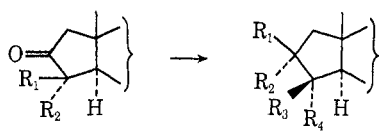
Received August 13, 1970

The successful preparation of a β-keto ester by the Dieckmann cyclization as a synthetic route to an *A*-nor steroid was first reported by Fuchs and Loewenthal in the cholestane series.[2] This synthesis was noteworthy for its specificity; of four possible isomers, with the carbomethoxyl substituted at either the 1 or 3 carbon with an α or β configuration, only one compound formed. The product was formed from the requisite diester, dimethyl 2,3-*seco*-5α-cholestan-2,3-dioate (**2**) (Scheme I), by treatment with potassium *tert*-butoxide in refluxing benzene.

SCHEME I



2



1a, $R_1 = CO_2Me$; $R_2 = H$
b, $R_1 = H$; $R_2 = CO_2Me$

3a, $R_1 = R_4 = H$; $R_3 = CO_2Me$; $R_2 = OH$
b, $R_2 = R_3 = H$; $R_4 = CO_2Me$; $R_1 = OH$

The original choice of configuration was made in favor of **1a** rather than **1b** for two reasons. It was shown that in the sodium borohydride reduction product, the hydroxy ester **3**, the hydroxyl and carbomethoxyl groups were trans with respect to one another. Then, by application of Klyne's principle of enantiomeric types,[3] the hydroxyl group was assigned as α; by inference, the carbomethoxyl group was β, and the structure was assigned as **3a**.

(1) (a) Abstracted in part from the M.S. thesis of B. V. P., Miami University, 1969. (b) Presented in part at the 2nd Central Regional Meeting of the American Chemical Society, Columbus, Ohio, June 3–5, 1970.
(2) B. Fuchs and H. Loewenthal, *Tetrahedron*, **11**, 199 (1960).
(3) W. Klyne, *J. Chem. Soc.*, 2916 (1952).