The ^{15}N–H coupling is increased by the introduction of electron-withdrawing substituents, whereas electron-donating substituents can be seen to have the reverse effect. Similar explanations are generally advanced to account for the difference in basicity between aromatic and aliphatic amines.[11]

A plot of the ^{15}N–H coupling constants for *meta* and *para* aniline-^{15}N derivatives against the appropriate Hammett substituent constants is shown in Figure 1.

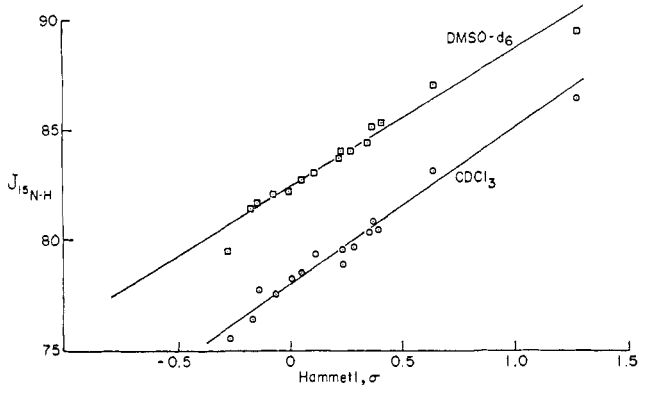

Figure 1.

Reasonably good linear correlations are observed using either DMSO-d_6 or $CDCl_3$ as solvent, although the fit is somewhat better in the former solvent case.

Effective conjugation of a mesomerically interacting substituent requires that both the amino group and the substituent be approximately coplanar with the benzene ring. This condition is subject to steric influences, and it is interesting to note that while 4-nitroaniline is correlated by the use of the σ^- value,[12] a poor correlation is obtained when this value is used in the case of 3,5-dimethyl-4-nitroaniline. Considerable evidence indicates that in the latter compound the nitro group is twisted out of the plane of the ring to the extent of 56°, leading to suppression of its resonance interaction with the amino group.[13] The observed coupling constant reflects this steric inhibition of resonance, and its correlation with the usual σ value for the nitro group seems justified.

Studies are in progress to determine the nitrogen-15 chemical shifts in these anilines.

Acknowledgment. This work was supported in part by a grant from The General Faculty Research Committee of The City College. We acknowledge the assistance of Mr. E. Sokoloski.

(11) P. A. S. Smith, "Open-Chain Nitrogen Compounds," Vol. I W. A. Benjamin, Inc., New York, N. Y., 1965, Chapter 3.
(12) H. H. Jaffé, *Chem. Rev.*, **53**, 250 (1953).
(13) J. P. Schaefer and T. J. Miraglia, *J. Amer. Chem. Soc.*, **86**, 64 (1964).

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Chemical Ionization Mass Spectrometry of Complex Molecules

Sir:

Despite the spectacular advances of mass spectrometry during the past decade, some of the basic problems attendant in its application still remain. Of these, perhaps the most serious from the point of view of the organic chemist is the failure on the part of many complex organic molecules to yield a stable molecular ion. This in turn is due to the instability of either the

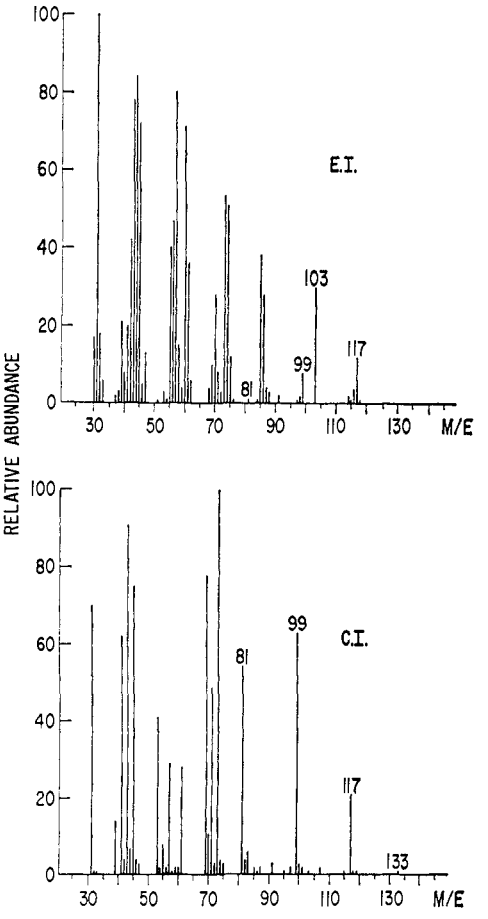


Figure 1. Mass spectra of 2-deoxy-D-ribose above *m/e* 30.

molecule itself or of the molecular ion which in normal electron impact (EI) mass spectrometry may be produced in a highly excited state. In an attempt to ameliorate the difficulties arising from the latter of these, we have investigated the potential of chemical ionization (CI) mass spectrometry in organic chemistry. The purpose of this communication is to report preliminary results of this work.

High-pressure mass spectrometry has been studied for many years by groups interested in ion–molecule reactions,[1] and recently Field, Munson, and coworkers have pioneered the technique of CI mass spectrometry.[2]

(1) "Ion-Molecule Reactions in the Gas Phase," Advances in Chemistry Series, No. 58, American Chemical Society, Washington, D. C., 1966.
(2) (a) F. H. Field, *Accounts Chem. Res.*, 1, 42 (1968); (b) M. S. B. Munson and F. H. Field, *J. Amer. Chem. Soc.*, **88**, 2621 (1966); (c) M. S. B. Munson and F. H. Field, *ibid.*, **88**, 4337 (1968); (d) M. S. B. Munson and F. D. Field, *ibid.*, **89**, 1047 (1967); (e) M. S. B. Munson and F. H. Field, *ibid.*, **89**, 4274 (1967); (f) F. H. Field, *ibid.*, **89**,

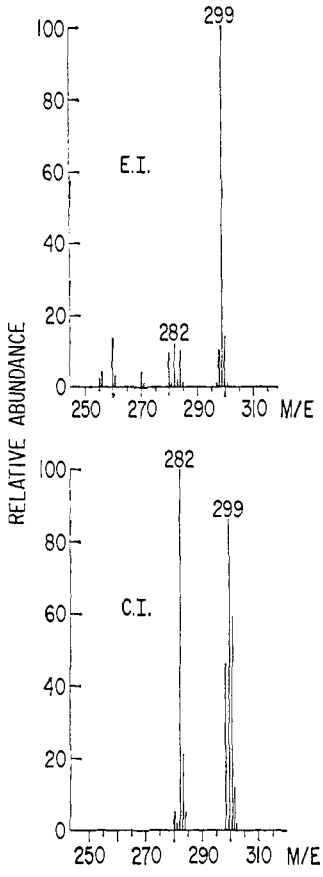


Figure 2. Mass spectra of codeine above *m/e* 250.

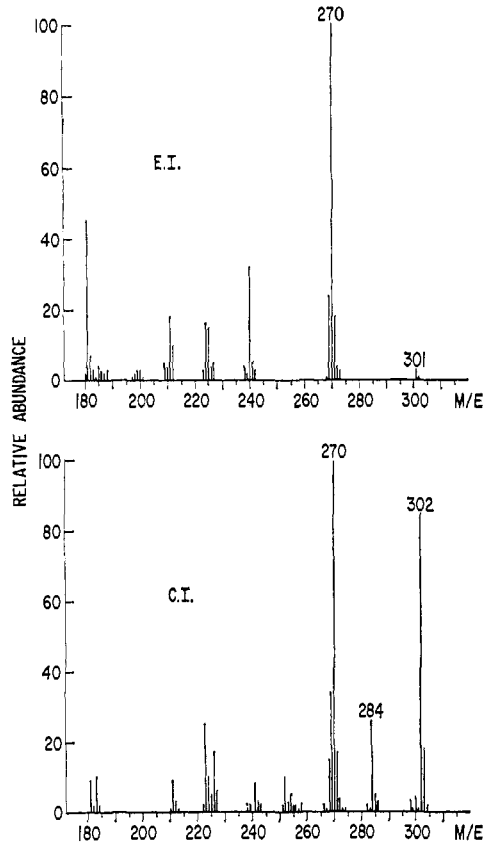


Figure 3. Mass spectra of crinamine above *m/e* 180.

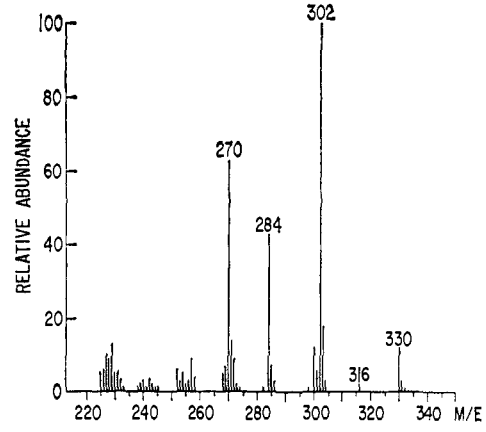


Figure 4. CI mass spectrum of haemanthamine above *m/e* 220.

In this method, a mixture of a reactant gas (>99%) and sample (<1%) is submitted to electron bombardment at pressures of about 1 mm. Collisions between electrons and sample will be relatively few, but the reactant gas will be extensively ionized. If methane is used as the reactant gas, the primary ions CH_4^+, CH_3^+, CH_2^+, and CH^+ will be formed, and at this pressure will enter into ion–molecule reactions with methane to give the secondary ions CH_5^+, $C_2H_5^+$, and $C_2H_3^+$ by well-established processes.[3] These secondary ions, upon collision with a sample molecule, will donate to it a proton or abstract from it a hydride ion, generating quasimolecular (QM^+) ions at *m/e* $(M + 1)^+$ or ions at *m/e* $(M - 1)^+$, respectively. The important distinction between this and conventional electron bombardment is that the amount of available energy in such collision-induced ionizations is of the order of 0–229 kcal (*i.e.*, <20 eV) in the case of methane, and the even-electron ion thus produced in the collision will not be energy rich, its lifetime being increased commensurately.

In order to operate at pressures of 1 mm the ionization chamber must be "tight" and the source housing and flight tube very adequately pumped so as to prevent interparticle collisions after the ions have been accelerated out of the ionization region. Such a source and pumping system has been designed for the MS-9 double-focusing mass spectrometer and is equipped with a direct insertion probe which was used for all samples discussed below.[4] All CI spectra were measured at an ionization chamber pressure of 0.8–5 mm and temperatures of 100–150°. The pressure was estimated from the reading of an ion gauge in the pumping line, a calibration with the ionization chamber pressure having been previously established. Alternatively, this source can be operated in the conventional EI mode by simply closing the reactant gas valve. The resulting system has been successfully operated at a resolving power of one part in 10,000 (10% valley), and on-line digitization and accurate mass measurement of ions have been carried out using as an internal reference *n*-dotriacontane, $C_{32}H_{66}$. "Metastable ions" are observed as usual.

5328 (1967); (g) F. H. Field, P. Hamlet and W. F. Libby, *J. Amer. Chem. Soc.*, **89**, 6035 (1967); (h) F. H. Field, M. S. B. Munson, and D. A. Becker, ref 1, p 167; (i) F. H. Field, *J. Amer. Chem. Soc.*, **90**, 5649 (1968).

(3) F. H. Field and M. S. B. Munson, *ibid.*, 87, 2289 (1965).

(4) This source was designed and built by Scientific Research Instruments Corporation, Baltimore, Md. A full description of the construction and performance of the source is to be published elsewhere.

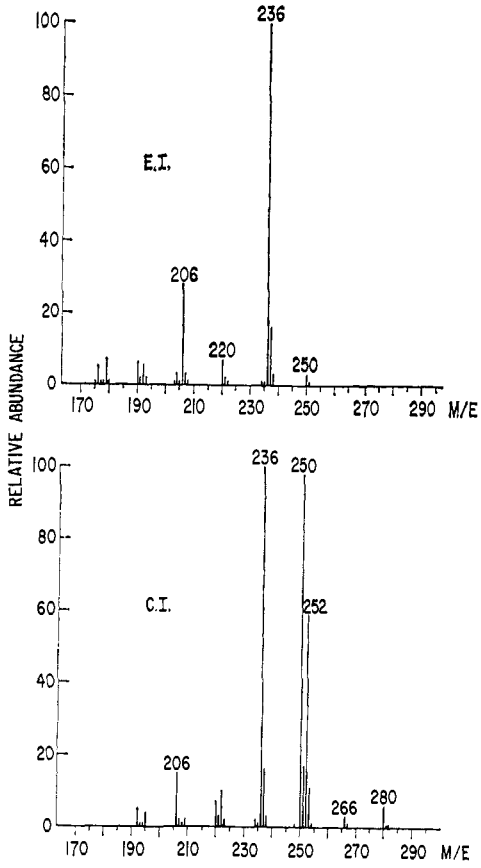


Figure 5. Mass spectra of O-methylpellotine above *m/e* 170.

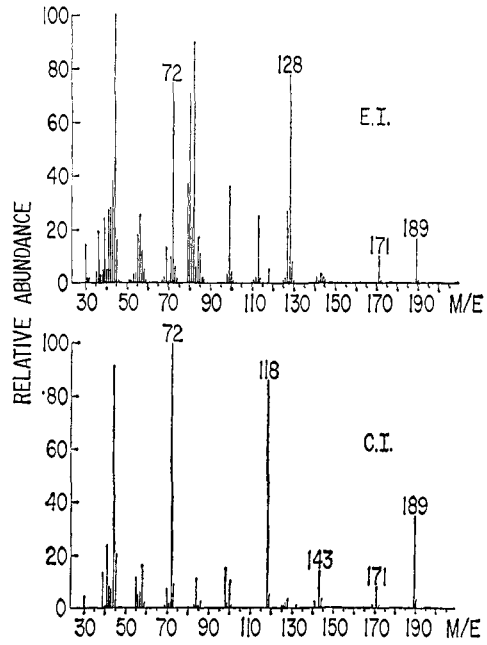


Figure 6. Mass spectra of alanylvaline above *m/e* 30.

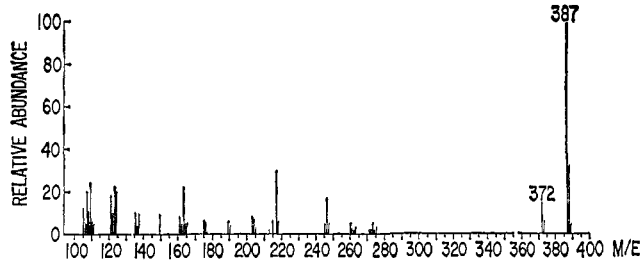
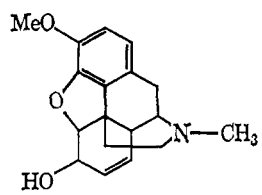


Figure 7. CI mass spectrum of cholestanone above *m/e* 100.

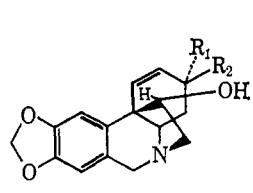
In Figure 1 are shown the EI and CI mass spectra of 2-deoxy-D-ribose. The CI spectrum shows a small ion at *m/e* 133 ($QM^+ - H_2$). Three major breakdown ions are at *m/e* 117, 99, and 81, corresponding to ($QM^+ - H_2O$), ($QM^+ - 2H_2O$), and ($QM^+ - 3H_2O$), respectively. "Metastable ions" at 83.8 (*m/e* 117 → *m/e* 99) and 66.3 (*m/e* 99 → *m/e* 81) are observed.

The success with which hydroxyl functions can be defined is repeated in the alkaloid field. Thus codeine, $C_{18}H_{21}NO_3$ (I), in the EI mode (Figure 2) gives a molecular ion at *m/e* 299, but the fate of the three oxygen atoms is not clear from the subsequent fragmentation. In the CI spectrum, however, the quasimolecular ion at *m/e* 300 (58%) is accompanied by an ion at *m/e* 282 ($QM^+ - H_2O$, 100%) and the appropriate "metastable ion" at *m/e* 266.1, thus confirming the presence in the molecule of a single hydroxyl group.

The EI spectra of the epimeric alkaloids crinamine (IIa) and haemanthamine (IIb) had commanded some interest[5] because the former loses methanol readily,



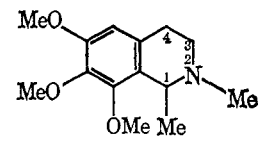
I



IIa, $R_1 = H$; $R_2 = OCH_3$
b, $R_1 = OCH_3$, $R_2 = H$

while the latter does not. In the CI spectra (Figures 3 and 4) both give an abundant ion at *m/e* 270, corresponding to loss of methanol from QM^+ which still occurs more readily in the crinamine case, and in addition both compounds now give an ion at *m/e* 284, corresponding to the loss of water from the quasimolecular ion at *m/e* 302.

A perennial problem in the interpretation of the mass spectra of tetrahydroisoquinoline alkaloids such as O-methylpellotine (III) has been to establish whether or not the intense ion in the molecular ion region repre-



III

sents $(M - 1)^+$ or $(M - 15)^+$ as the molecular ion usually breaks down completely by loss of the substituent or the proton at C-1 by β fission. Thus the EI spectrum of III (Figure 5) has a small ion at *m/e* 250, and the base peak of the spectrum is at *m/e* 236. In the CI spectrum of the same compound, however, the quasimolecular ion at *m/e* 252 (59%) is accompanied by ions at *m/e* 250 (98%) and 236 (100%), reflecting equally facile cleavage of the C-1 hydrogen and methyl group. The small peaks at *m/e* 266 and 280 represent ($M + CH_5^+ - H_2$) and ($M + C_2H_5^+$), respectively.

(5) A. M. Duffield, R. T. Aplin, H. Budzikiewicz, C. Djerassi, C. F. Murphy, and W. C. Wildman, *J. Amer. Chem. Soc.*, 87, 4902 (1965).

In a different area, the CI spectrum of free alanylvaline was studied. This spectrum differs quantitatively but not qualitatively from the EI spectrum (Figure 6). Both give a quasimolecular ion, and the important sequence-determining ion at *m/e* 72 $(CH_3CHNH_2CO)^+$ is present in both spectra, being the base peak in the CI spectrum. The intense ion at *m/e* 118 is presumably protonated valine $(C_5H_{12}NO_2)^+$.

Finally, the CI spectra of some steroidal ketones were studied. In this series, the formation of a quasimolecular ion at *m/e* (M + 1) as the base peak of the spectrum appears to be general, but the remarkable variation in the abundance of the peak corresponding to loss of water (0% in cholestan-3-one (Figure 7), 60% in androstan-17-one) suggests that location of the carbonyl group in the steroid nucleus may be determined in this way.

The foregoing examples are encouraging in that they confirm the original supposition that somewhat simpler fragmentations might prevail in CI mass spectrometry. The sensitivity of the spectrometer for important ions in the molecular ion region is increased by its being operated in CI mode, and these observations, taken together, permit the conclusion that the method merits further study, such as is being undertaken in these laboratories.

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A General Biochemical Synthesis of Oxygenated Prostaglandins E

Sir:

The prostaglandins are a family of compounds derived biosynthetically from unsaturated fatty acid precursors such as 8,11,14-eicosatrienoic or 5,8,11,14-eicosatetraenoic acids.[1,2] These substances have a widespread distribution in the body and exhibit a multitude of pharmacological effects.[3] Until now, no oxygenated prostaglandin E (PGE) has been synthesized or isolated from natural sources.[4] We herein describe a synthesis of 11α,15-(*S*)-dihydroxy-9,18-dioxo-5-*cis*,13-*trans*-prostadienoic acid (18-oxo-PGE_2), and 11α,15-(*S*)-dihydroxy-9,19-dioxo-5-*cis*,13-*trans*-prostadienoic acid (19-oxo-PGE_2). The principle of this method involves microbiological hydroxylation of 5,8,11,14-eicosatetraenoic acid; the resulting oxygenated unsaturated fatty acids or their derivatives can then be cyclized by exposure to bull seminal vesicle microsomes (BSVM) to yield the desired oxygenated prostadienoic acid derivatives.

Exposure of 5,8,11,14-eicosatetraenoic acid (I) (1 g) to *Ophiobolus graminis* afforded two polar products, which were characterized as 18ϵ-hydroxy-5,8,11,14-eicosatetraenoic acid (II) (200 mg) and 19ϵ-hydroxy-5,8,11,14-eicosatetraenoic acid (III) (170 mg) on the basis of the following data. Treatment of II with diazomethane afforded the methyl ester, whose mass spectrum showed a parent ion peak at *m/e* 334. Hydrogenation of the methyl ester over PtO_2, followed by chromic acid oxidation, resulted in the formation of the saturated keto ester, IV, mp 62°. Its mass spectrum exhibited the parent ion peak at *m/e* 340 with peaks at *m/e* 311 (M − 29) and *m/e* 269 (M − 71), corresponding to α and β cleavages as indicated. The methyl ester of III also showed a parent ion peak at *m/e* 334, consistent with the empirical formula, $C_{21}H_{34}O_3$. By a similar sequence of reactions, the saturated keto ester V, mp 56°, was obtained. Its nmr[5] spectrum exhibited bands at τ 8.72 (30 H, singlet, CH_2), 7.97 (3 H, singlet, $CH_3C{=}O$), 7.78 (4 H, multiplet, CH_2 adjacent to carbonyl), 6.4 (3 H, singlet, $-COOCH_3$). Its mass spectrum displayed a parent ion at *m/e* 340, and the characteristic peak[6] at *m/e* 283 (M − 57), corresponding to β cleavage as indicated, α cleavage of terminal methyl ketones being of minor significance.[6]

Although II and III could be cyclized to their corresponding prostadienoic acid derivatives by BSVM, the yields (<10%) were low. Furthermore, the 18- and 19-acetoxy or methoxy eicosatetraenoic acid derivatives were not cyclized by BSVM. On the other hand, both 18-oxo-5,8,11,14-eicosatetraenoic acid (VI) and 19-oxo-5,8,11,14-eicosatetraenoic acid (VII) obtained by Jones oxidation from II and III, respectively, were converted into their prostadienoic acid derivatives. In a typical experiment, VI (345 mg) was incubated with BSVM (14.3 g) in 0.05 *M* phosphate buffer, pH 7.5, in the presence of hydroquinone (207 mg) and glutathione (690 mg) for 5 hr at 37°. After the usual work-up,[7] followed by successive column chromatography on silicic acid, reverse-partition chromatography,[8] and silicic acid, 40 mg of 18-oxo-PGE_2 (VIII) was obtained. Alkaline treatment of VIII afforded a compound with absorption maxima at 278 mμ,[9] reminiscent of the prostaglandin B type chromophore, and at 330 mμ. Its nmr spectrum revealed signals at τ 8.96 (3 H, triplet, J = 3.5 cps), 8.26 (quartet, J = 3.5 cps), 5.88 (2 H, multiplet), 4.45 and 4.35 (6–7 H), characteristic of the E_2 type of prostaglandin.[10] The mass spectrum of VIII gave a parent ion at *m/e* 348 (M − 18) and *m/e* 330 (M − $2H_2O$), corresponding to the loss of the 11 and 15 hydroxyl groups; *m/e* 204 (330 − 126), a McLafferty cleavage at C-8 with loss of the carboxylic side chain; *m/e* 273 (330 − 57) and *m/e* 258 (330 − 72) represent α and β cleavages as indicated. 15-(*S*)-Hydroxy-9,18-dioxo-10-prostaenoic acid methyl ester (X) was prepared from VIII by methylation, hydrogenation over PtO_2,

(1) D. A. Van Dorp, R. K. Beerthuis, D. H. Nugteren, and H. Vonkeman, *Biochim. Biophys. Acta*, **90**, 204 (1964).
(2) S. Bergstrom, H. Danielson, and B. Samuelsson, *ibid.*, **90**, 207 (1964).
(3) S. Bergstrom, L. A. Carlson, and J. R. Weeks, *Pharmacol. Rev.*, **20**, 1 (1968).
(4) 19-Hydroxyprostaglandins A_1, A_2, B_1, B_2 were isolated from human seminal plasma (M. Hamberg and B. Samuelsson, *J. Biol. Chem.* **241**, 257 (1966)).
(5) Nuclear magnetic resonance spectra were determined on a Varian Associates recording spectrometer (A-60A) at 60 Mc in deuterated chloroform. Chemical shifts are reported in τ values (parts per million) (G.V.D. Tiers, *J. Phys. Chem.*, **62**, 1158 (1958)).
(6) R. Ryhage and E. Sternhagen, *Arkiv Kemi*, **37**, 545 (1960).
(7) E. G. Daniels, J. W. Hinman, B. A. Johnson, F. P. Kupiecki, J. W. Nelson, and J. E. Pike, *Biochem. Biophys. Res. Commun.*, **21**, 413 (1965).
(8) A. Norman, *Acta Chem. Scand.*, **7**, 1413 (1953).
(9) The extinction coefficient could not be measured accurately since the λ_{max} compound rapidly decomposed to another product with λ_{max} at 237 mμ in the presence of base.
(10) P. W. Ramwell, J. E. Shaw, G. B. Clarke, M. F. Grostic, D. G. Kaiser, and J. E. Pike, *Progr. Chem. Fats Lipids*, **9**, 233 (1968).