compounds and III in aqueous solutions is structure dependent. The presence of polar functional groups (*e.g.*, hydroxyl, amino, carboxylic, and sulfonic groups) in unionized forms facilitates interaction with the polymer.

The thermodynamic driving force for dissolution enhancement in prostanglandin dispersions appeared to derive from molecular interaction (in this case, adsorption) of the prostaglandin with III. This adsorption enabled the breaking up of the crystal lattice of the prostaglandin, thus requiring less energy input for dissolution. When no adsorption took place, the prostaglandin probably precipitated out by itself on evaporation of the acetonitrile, existing as separate particles unassociated with the polymer. These precipitates would essentially have the same physical characteristics as the physical mixtures and, therefore, would not be expected to show enhancement in dissolution.

It appears feasible to predict whether a certain prostaglandin may benefit in dissolution from dispersion formation with III. When a prostaglandin interacts with III, as indicated by significant adsorption, the dispersion formed probably will exhibit an increased dissolution rate. Prostaglandins that show poor interaction with III probably will not improve their dissolution rates through dispersion formation.

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Formation of a Cyclic Derivative of Ethacrynic Acid with Diazomethane

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Abstract □ Samples of ethacrynic acid were treated with methanol–hydrochloric acid or with diazomethane. GLC and mass spectrometric analysis indicated that the methanol–hydrochloric acid reaction gave the expected methyl ester, whereas diazomethane treatment gave a compound containing an additional 14 mass units. Accurate mass measurement and PMR and IR spectra showed that this product was a cyclic derivative of the methyl ester of ethacrynic acid, methyl 4-(2,3-dihydro-4-ethyl-5-furyl)-2,3-dichlorophenoxyacetate. Either derivatization method can be used for development of an assay for ethacrynic acid.

Keyphrases □ Ethacrynic acid—reaction with methanol–hydrochloric acid or diazomethane, PMR, IR, and GLC–mass spectral analyses of products □ GLC—analysis, derivatives of ethacrynic acid □ Derivatization—ethacrynic acid with methanol–hydrochloric acid or diazomethane, PMR, IR, and GLC–mass spectral analyses of products □ Diuretics—ethacrynic acid, reaction with methanol–hydrochloric acid or diazomethane, PMR, IR, and GLC-mass spectral analyses of products

Ethacrynic acid (I) is a potent, orally active diuretic (1–4) for which a suitable GLC procedure was required for investigating its pharmacokinetic profile in humans. Previously reported analytical procedures involved the administration of ^{14}C-I followed by a tedious workup using TLC or column chromatography (4, 5).

Ethacrynic acid is not amenable to direct GLC analysis, and an approach was to convert it into its methyl ester either by treatment with diazomethane or by reaction with

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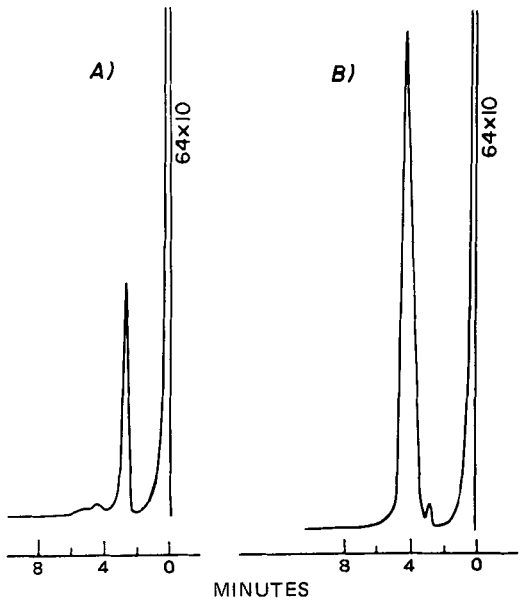


Figure 1—*Typical chromatogram of I when reacted with methanol–hydrochloric acid (A) and when treated with diazomethane (B).*

methanol–hydrochloric acid prior to GLC. The methylation reactions of diazomethane have been reviewed (6–9). The diazomethane treatment of I gave a major product with a different retention time on GLC analysis than that obtained from the methanol–hydrochloric acid reaction. This paper reports characterization of the two products as determined by GLC–mass spectrometry, accurate mass measurement, PMR spectroscopy, and IR spectrophotometry.

EXPERIMENTAL

Reagents and Chemicals—Methanol[2], hydrochloric acid[3], and ether[4] were obtained commercially. Diazomethane was generated using a commercially available kit[5].

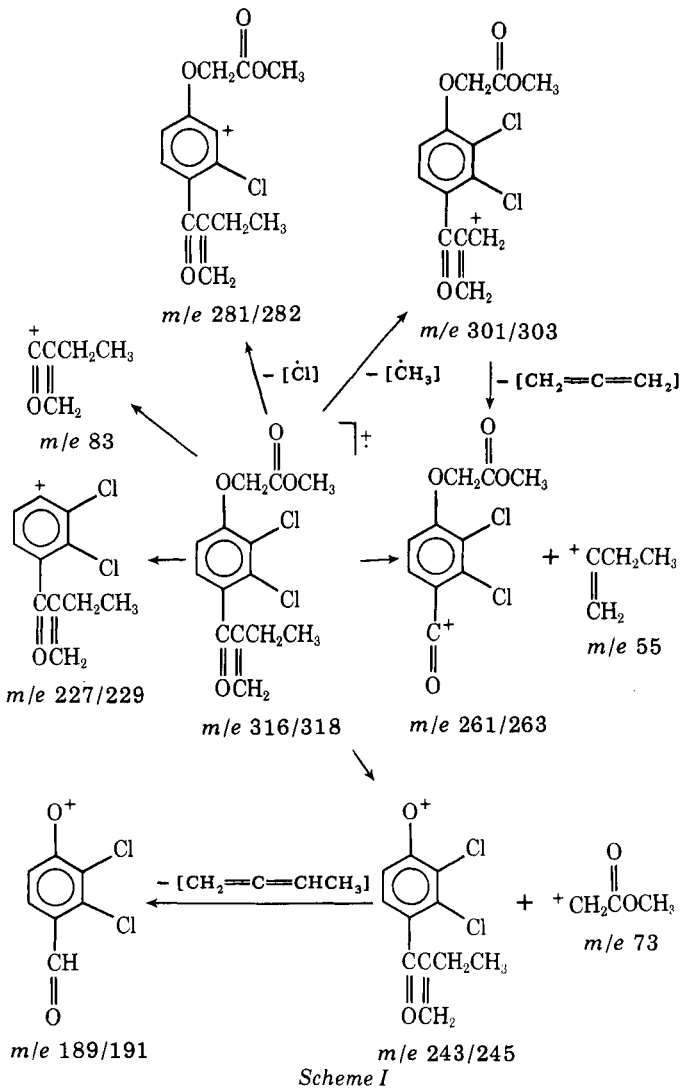
GLC—The gas chromatograph[6] was equipped with a flame-ionization detector. The column was coiled glass tubing, 1.83 m (6 ft) long × 2 mm i.d., packed with 2% phenyl methyl silicone fluid[7] (OV-25) on acid-washed, dimethyldichlorosilane-treated, high performance Chromosorb W[7] support (80–100 mesh).

The column was conditioned by maintaining the oven at 300° for 18 hr with a low nitrogen flow. Operating temperatures were: injection port, 250°; column, 225°; and detector, 250°. The nitrogen flow rate was 60 ml/min. Hydrogen and compressed air flow rates were adjusted to give the maximum response.

GLC–Mass Spectrometry—GLC–mass spectrometry was carried out on a mass spectrometer[8] coupled to a gas chromatograph[9] through a two-stage jet separator. The ionization potential was 70 ev. The GLC column and conditions were the same as for direct GLC.

For accurate mass measurement, the sample of reacted product was collected from the detector at its appropriate retention time after extinguishing the flame. The accurate mass was determined on a high-resolution mass spectrometer[10] equipped with a direct insertion inlet and operating at 70 ev with a source temperature of 160°.

PMR—All PMR spectra were recorded[11] at 60 MHz at ambient temperature, using deuterated chloroform as the solvent and tetramethylsilane as the internal standard.

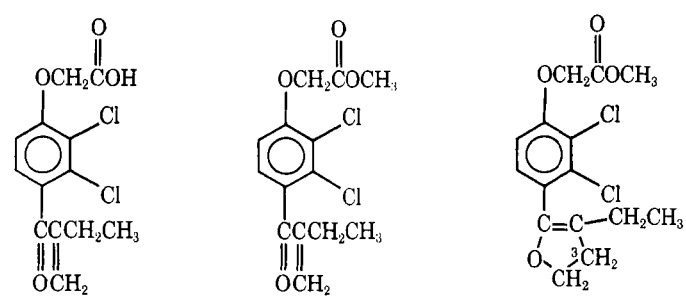


Scheme I

IR—The IR spectra were recorded[12] using solutions in chloroform at ambient temperature.

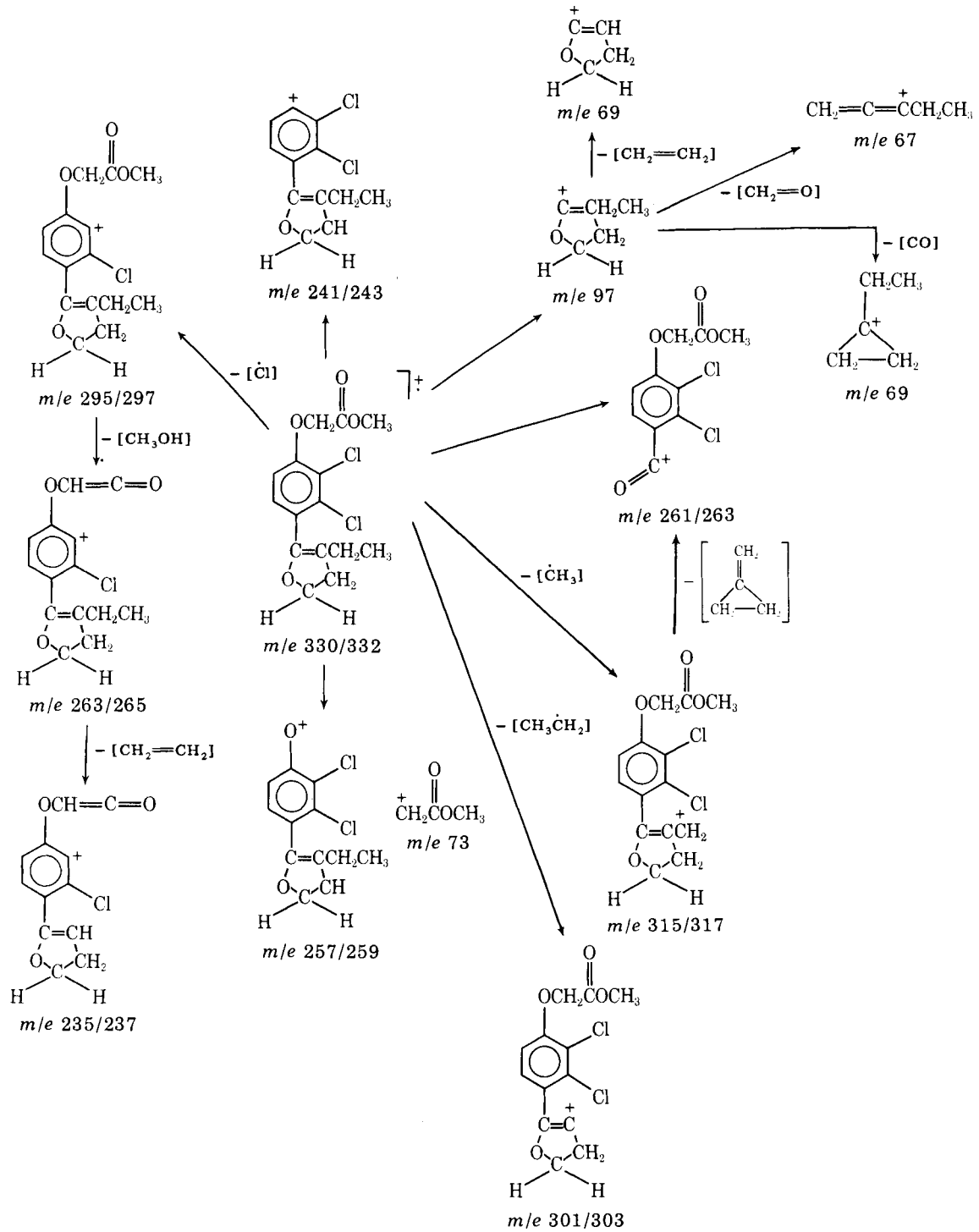
Methylation—Methyl derivatives were prepared by treating aliquots (0.1–100 mg) of solid I with an excess of ethereal diazomethane[5] at room temperature for 15 min with occasional mixing. Excess diazomethane was evaporated at room temperature under a stream of dry nitrogen.

Alternatively, aliquots of solid I (0.1–100 mg) were treated with 1–5 ml of a methanolic solution of concentrated hydrochloric acid [concentrated hydrochloric acid–methanol (1:4)] for 1 hr at 65°. These solutions were evaporated to dryness at 65° under a stream of dry nitrogen. The residues were dissolved in 0.5 ml of 10 *N* NaOH, extracted[13] with 5 ml of ether for 10 min, and then centrifuged for 10 min at 2500 rpm. The ether layers were transferred to other tubes. The extracts were evaporated to a volume of 100 μl at 45° under a stream of dry nitrogen.



I II III

[2] Burdick and Jackson Laboratories, Muskegon, Mich.
[3] Baker analyzed reagent, Canlab, Montreal, Quebec, Canada.
[4] Anhydrous, Mallinckrodt Chemical Works, Montreal, Quebec, Canada.
[5] Catalog No. 710,025-0, Aldrich Chemical Co., Milwaukee, Wis.
[6] Model 3920, Perkin-Elmer Canada.
[7] Chromatographic Specialties, Brockville, Ontario, Canada.
[8] Hitachi Perkin-Elmer model RMU 6L.
[9] Model 990, Perkin-Elmer Canada.
[10] Varian MAT 311, Varian of Canada, Georgetown, Ontario, Canada.
[11] Varian A-60A, Varian of Canada, Georgetown, Ontario, Canada.
[12] Unicam SP-1000, Canlab, Montreal, Quebec, Canada.
[13] Roto-Rack, Fisher Scientific Co., Montreal, Quebec, Canada.



Scheme II

Aliquots (1–2 μl) from either procedure were injected into a gas chromatograph equipped with a flame-ionization detector.

RESULTS AND DISCUSSION

GLC examination of the products obtained from the reaction of I with methanol–hydrochloric acid showed a major peak (97% based on peak area) and a minor peak (≃2% based on peak area) with retention times (R_t) of 2.8 and 4.3 min, respectively (Fig. 1A). The GLC–mass spectrum (Fig. 2A) of the compound giving rise to the major peak showed a molecular ion at *m/e* 316/318 and other diagnostic ions at *m/e* 301/303, 281/283, 261/263, 243/245, 227/229, 189/191, 89, 73, and 55. The formation of these ions is postulated in Scheme I.

The PMR spectrum of this product did not exhibit a singlet at 9.97 ppm for the carboxylic acid proton as observed in the spectrum of I. The only other difference between the spectra was the presence of a singlet (3H) at 3.83 ppm in the spectrum of the product. It was concluded, therefore, that the product was the expected methyl ester (II) of ethacrynic acid.

GLC examination of the product obtained by the reaction of diazomethane with I also gave a major peak (99% based on peak area) but with a retention time of 4.3 min (Fig. 1B) under the same conditions. The GLC–mass spectrum of the compound giving rise to this peak (Fig. 2B) showed an ion of highest mass at *m/e* 330/332 and other ions at *m/e* 315/317, 301/303, 295/297, 263/265, 261/263, 257/259, 241/243, 235/237, 189/191, 97, 73, 69, and 67.

GLC–mass spectral analysis of the compound giving rise to the minor peak (R_t 4.3 min, Fig. 1A) in the methanol–hydrochloric acid reaction of I gave a mass spectrum identical to that shown in Fig. 2B (molecular ion at *m/e* 330/322 and other ions).

The addition of 14 mass units over the molecular weight of II suggested

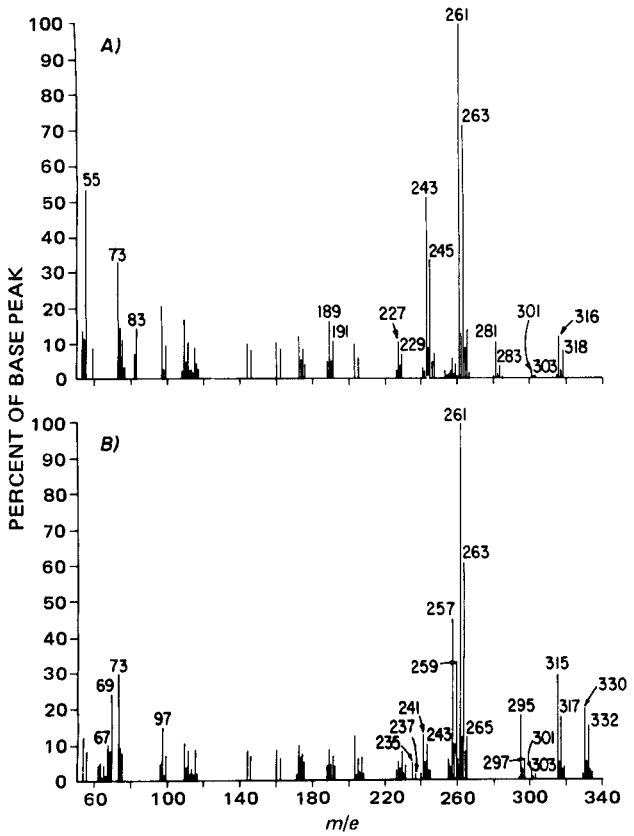


Figure 2—*GLC–mass spectrum (normalized) of the major product obtained from methanol–hydrochloric acid reaction of I (A) and treatment of I with diazomethane (B).*

the addition of an extra methylene unit to the molecule. This structure was supported by an accurate mass determination for $C_{15}H_{16}Cl_2O_4$ from diazomethane-treated I (calc. for $C_{15}H_{16}{}^{35}Cl_2O_4$: 330.0426, found 330.0426; calc. for $C_{15}H_{16}{}^{35}Cl^{37}ClO_4$: 330.0397, found 330.0396).

The IR spectrum of the diazomethane-treated product retained the band at 1765 cm^{-1} (C=O, ester carbonyl), but the strong band at 1670 cm^{-1} (C=O, keto function) in the spectrum of II was replaced by a weaker, broader band at 1690–1710 cm^{-1}.

Significantly, comparison of the PMR spectrum of the diazomethane-treated product with that of II showed the absence of the two singlets at 5.62 and 5.97 ppm, corresponding to the two vinylic protons of II. Instead, the spectrum contained a triplet (2H, J = 8 Hz) centered at 4.57 ppm and a complex multiplet (4H) spread between 1.32 and 2.57 ppm, arising from overlapping signals due to the methylene protons of the ethyl group and two vinyl proton multiplets (Fig. 3).

These IR, PMR, and accurate mass measurement data are consistent with the cyclic Structure III for the diazomethane-treated product. The major ions observed in the mass spectrum can be explained on the basis of this structure as illustrated in Scheme II. The medium intensity band at 1690–1710 cm^{-1} in the IR spectrum is consistent with the ν C=C of a cyclic vinyl ether. An exocyclic cyclopropano keto function would also absorb in this region (10), but the observed band was weaker and broader

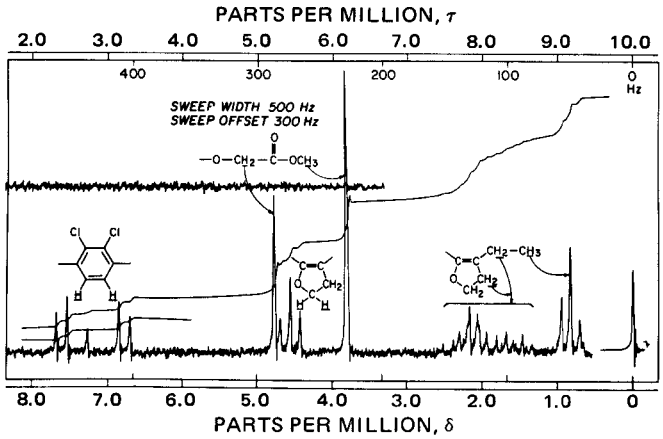


Figure 3—*PMR spectrum (deuterochloroform solution) of the major product obtained from diazomethane treatment of I.*

than the ν C=O of a keto function. Furthermore, there was no evidence of cyclopropano protons in the PMR spectrum.

It was concluded that the major product of the reaction of diazomethane with ethacrynic acid is methyl 4-(2,3-dihydro-4-ethyl-5-furyl)-2,3-dichlorophenoxyacetate (III). An analogous 1,4-addition of methylene occurs when certain α,β-diketones, such as benzil and phenanthraquinone, are allowed to react with diazomethane in the presence of methanol (11).

Either derivatization method appears to be suitable for the development of an assay for I, but the methanol–hydrochloric acid route avoids the hazards implicit in the use of diazomethane.

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