A detailed description of the ^{13}C-NMR technique used, complete tables for all acids studied and all ^{13}C-shifts, and references for the preparation of the α,β-unsaturated acids will be given elsewhere.

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An Apparatus for Displacement Electrophoresis

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During the last three years, several papers [1–7] have been published on an electrophoretic technique of separating ions of the same charge-sign but different mobilities. According to this technique, originally suggested by Kendall [8] and later named "displacement electrophoresis" by Martin,[3] a sample of the ions to be separated is placed, in the direction of migration, behind a more mobile ion than any of the same charge-sign in the sample, and in front of a less mobile ion. On passage of current, the ions in the sample will arrange themselves, in the order of decreasing mobility, into a system of consecutive zones, separated by sharp moving boundaries. The length of a zone is proportional to the amount of the ion in the sample. The proportionality constant is a function of the mobility of the sample ion and the concentration and mobility of the fast ion.

At this laboratory, work has been going on since 1964 to develop, according to the principles above, an apparatus for quantitative analysis of fatty acid mixtures. As, among other things, the ways of introducing the sample and detecting the moving boundaries with the apparatus constructed here are quite different from those in the literature cited, a short description of its design and function is given below together with some results obtained with it.

The electrophoresis apparatus consists basically of a U-tube of glass (Fig. 1) with attached electrode vessels (E_1, E_2). The U-tube is surrounded by a cooling mantle of glass. The electrodes are platinum wires, dipping down about 1 cm into the solutions. To facilitate the introduction of the sample, a special valve (V) is inserted between the narrower leg (U_1, ϕ 4 mm) of the U-tube and E_1.

The valve consists of three pieces of Perspex, two of which are fixed (A and B in Fig. 2) and one (C) is movable between two end positions by means of an oblong hole (H) and a screw (S). In each piece,

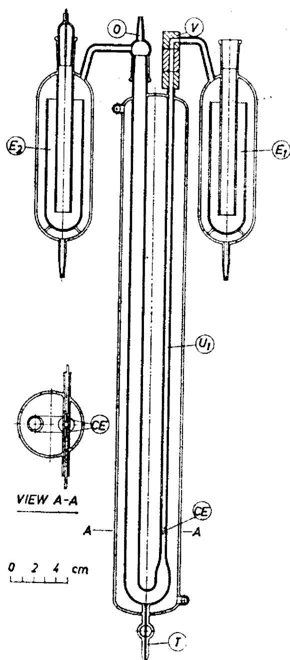

Fig. 1. Electrophoresis apparatus. For explanation, see text.

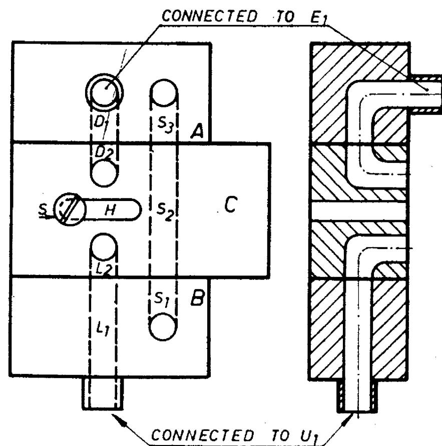

Fig. 2. Valve for introduction of sample. For explanation, see text.

channels (ϕ 4 mm) have been drilled (L_1, L_2, D_1, D_2, and S_1-S_3 in Fig. 2).

With C in the end position shown in Fig. 2, the U-tube and the channels L_1 and L_2 are filled through T (Fig. 1) with the solution containing the fast ion (the leading solution). Further, E_1, D_1, and D_2 are filled with the solution of the slow ion (the displacing solution) and S_1-S_3 with the sample solution. Finally, E_2 and its connection to the U-tube are filled with the leading solution. The air then escapes through O. After the menisci in E_1 and E_2 have been adjusted to the same level and the electrodes inserted, C is moved to its opposite end position. This brings S_2 in connection with L_1 and D_1.

Current (2.0 mA) from a constant-current power supply (max. 5 mA, 15 kV, built at the Institute by Mr. M. Geiser) is now sent through the U-tube in such a direction as to give a downward migration of the system of moving boundaries. The current is left on until all the boundaries have passed a pair of conductance electrodes (CE in Fig. 1).

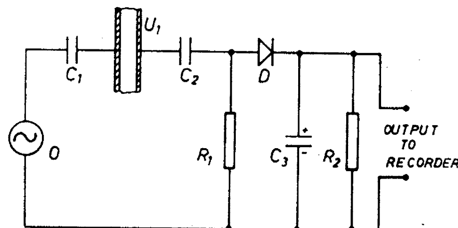

Fig. 3. Schematic diagram of the alternating current circuit. U_1: part of the electrophoresis tube (*cf.* Fig. 1). C_1, C_2: condensers (0.025 μF, 15 kV). O: oscillator (Hewlett-Packard 200 CD, operated at 20 kc). R_1, R_2: resistors (20 resp. 270 kohm). C_3: condenser (6.4 μF, 25 V). D: diode (1 N 5059). The recorder was a Moseley Autograph, 7101 A.

The conductance electrodes are capacity coupled in an a.c. circuit (Fig. 3). Since the conductivity of each zone is constant and lower than that of the preceding zone, the recorder will plot a series of steps as the boundaries pass the electrodes (Fig. 4). The height of a step (H in Fig. 4) is characteristic of the mobility of the species of ion in the corresponding zone and the length (L) of the step is proportional to

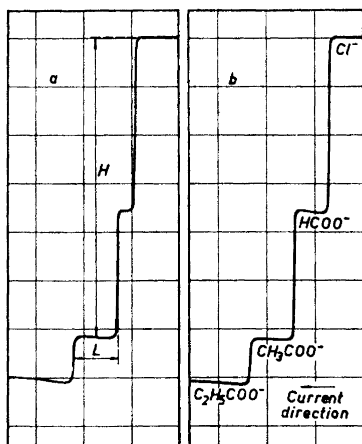

Fig. 4. Displacement electropherograms of mixtures of NaOOCH and $NaOOCCH_3$: a) 0.005 m NaOOCH, 0.01 m $NaOOCCH_3$, b) 0.01 m NaOOCH, 0.01 m $NaOOCCH_3$. Leading solution: 0.01 m NaCl. Displacing solution: 0.02 m $NaOOCC_2H_5$. Stabilizer: 2 % hydroxyethylcellulose. Final zone lengths L (mm) of NaOOCH: 9.5 (a), 18.6 (b); of $NaOOCCH_3$: 23.4 (a), 23.2 (b).

The conductance within a given zone decreases in the direction of migration. This is reasonable considering the temperature-profiles across moving boundaries in a narrow tube as measured (and calculated) by Everaerts[7] and the fact that conductance increases with temperature.

the amount of that ion present. The latter is also verified by the figures of Table 1. The record thus gives both qualitative and quantitative information about the sample.

Table 1. Variation of final zone length with amount of sample ion ($HCOO^-$) introduced. Leading solution: 0.01 m NH_4Cl. Displacing solution: 0.002 m NH_4OOCCH_3. Stabilizer: 0.16 % agarose.

Amount of sample ion (μmole)	Zone length (mm)
0.50	7.0
0.75	10.3
1.00	14.0
2.00	26.9

The conductance electrodes were constructed and inserted into the glass wall of U_1 essentially as described by Lorimer, Graham, and Gordon.[9] The a.c. circuit is a modification (by Mr. S. Flodin at this Institute) of their circuit.

One necessary condition for obtaining useful electropherograms is that the solutions in the separation tube are stabilized against convection. Otherwise the fact that in most cases one or other of the developed zones is denser than the one just below will spoil the separation. To find a proper stabilizer was not easy. Several polymers, both water-soluble and water-insoluble ones, were tried. Water-insoluble media as cellulose and plastic powder (Pevikon) proved to be inapplicable since they could not be uniformly packed in the narrow tube U_1. Moreover, the porosity of such a packing and thereby the mobility of an ion species changed in time. Three different water-soluble polymers were tried: ethyl-hydroxyethylcellulose (Modocoll EK 600, made by Mo och Domsjö, Sweden), agarose (prepared according to Hjertén [10]), and hydroxyethylcellulose (Schuchardt, Germany). To suppress convection sufficiently, concentrations of 1.5, 0.16, and 2.0 %, respectively, were required.

Solutions of Modocoll were found very difficult to wash away from glass walls. Moreover, this polymer as well as agarose gave rise to artefacts in the electropherograms. More steps than expected were obtained with known samples. A probable cause of this is presence of ionic impurities in the polymers used. It was not possible, however, to get rid of the artefacts by purification of the polymers. Small disturbances in the electropherograms were obtained also after prolonged electrodialysis of the polymer solutions before adding the electrolytes.

The hydroxyethylcellulose, on the other hand, when deprived of its small content of sodium acetate, worked without any complications. This was accomplished by stirring the polymer solution twice with an excess of a mixed-bed ion exchanger (ratio 5:100) as suggested by Everaerts.[7]

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Structures of Urinary Metabolites of Prostaglandin $F_{2\alpha}$ in the Rat

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One of the urinary metabolites of prostaglandin $F_{1\alpha}$ in the rat has been found to be dinor-PGF$_{1\alpha}$.[1] Recently the main urinary metabolites of prostaglandin E_2 in guinea pig and man have been identified as 5β,7α-dihydroxy-11-keto-tetranorprostanoic acid and 7α-hydroxy-5,11-diketo-16-carboxy-tetranorprostanoic acid, respectively.[2,3]

9β-^{3}H- and/or 17,18-^{3}H-prostaglandin $F_{2\alpha}$ (3.2 μC/μmole; 1.4 μmole per rat) was administered intravenously to female rats. The urine was collected during 24 h, acidified to pH 3 and extracted with butanol (50 % of administered radioactivity was recovered). Separation of the extract by reversed phase partition chromatography[4] gave four peaks of radioactivity at retention volumes 150 ml (peak I), 300 ml (peak II), 700 ml (peak III), and 1300 ml (peak IV), respectively (system C-38, 45 g of hydrophobic Hyflo-Supercel).

After treatment with diazomethane the materials in peak I and III were each separated on a silicic acid column. In both chromatograms two peaks appeared, compound Ia and Ib eluted with methanol/chloroform 2/98 and 8/92, respectively, and compound IIIa and IIIb eluted with ether/hexane 60/40 and 80/20, respectively.

Compound IIIa. The *O*-methyloxime acetyl (MO-AC) derivative was prepared[5] and chromatographed on a Barber-Colman Gas Chromatograph model 500 with simultaneous registration of mass and radioactivity using a 1 % Se-30 column at 220°C. One peak appeared at 21.9-C.[6] This C-value is consistent with a C_{16} prostaglandin with two acetoxy groups and one methoxime group.

Mass spectra of the MO-Ac and acetyl derivatives were obtained on an LKB 9000 A gas chromatograph-mass spectrometer. Some relevant ions are listed in Table 1 (acetyl derivative) and Fig. 1 (MO-Ac derivative). The molecular ions were seen in both spectra (m/e 398 and m/e 427) and eliminations characteristic for methyl ester acetyl derivatives (15, 31, 32, 59, 60, and 73) are easily identified. These data strongly support the structure shown in Fig. 2. The loss of 56 in both spectra is due to β-cleavage at the keto or methoxime group and transfer of hydrogen to the charge retaining ion, while α-cleavage on each side of the keto group of the acetyl derivative causes elimination of 71 and 99 mass units. The ion at m/e 156 is due to the ionized side chain attached to C-8. The mass spectrum of the MO-Ac derivative deuterated in the methoxime group supported the eliminations proposed above.

Compound Ia. The MO-Ac derivative had a C-value of 24.9 on GLC and some mass spectral data are listed in Table 1 and Fig. 1. The eliminations involving 100 mass units are due to β-cleavage at the methoxime group and it is shown in Fig. 1 that this gives rise to ions common for metabolites IIIa and Ia. Therefore the higher molecular weight of Ia must be due to a carbomethoxy group in the eliminated fragment. The GLC and mass spectral data strongly suggest the structure in Fig. 2. Mass spectra of MO-Ac derivatives deuterated in the methoxime group or the acetyl groups confirmed the structure and proposed eliminations.

Compound Ib. On GLC the MO-Ac and MO-TMS (*O*-methyloxime trimethyl silyl ether) derivatives gave C-values of 25.7 and 24.1,