Journal of Chromatography, 309 (1984) 243—259
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2144

CHARACTERIZATION OF ARTEFACTS PRODUCED BY TREATMENT OF ORGANIC ACIDS WITH DIAZOMETHANE

SONJA BAUER, MANFRED NEUPERT and GERHARD SPITELLER*

Lehrstuhl für Organische Chemie I, Universität Bayreuth, Postfach 3008, Universitätsstrasse 30, D-8580 Bayreuth (F.R.G.)

(Received February 15th, 1984)

SUMMARY

When α,β-unsaturated acids and α-keto acids react with diazomethane not only are the corresponding methylates produced, but also diazomethane is added to the C=C double bond or to the oxo group. The gas chromatographic and mass spectral behaviour of these undesired products and some further artefacts produced in the hot inlet lines of a gas chromatograph are described. The mass spectra and retention indices allowed the structural assignment of several "unknown" compounds found previously in the methylated acid fraction of urine. A detailed analysis of the reaction of α-oxo acids with diazomethane revealed that, besides the already known oxirane methyl esters, homologous esters are also produced by an insertion reaction.

INTRODUCTION

Acidic compounds occurring in biological fluids are obtained either by extraction [1—3] or ion-exchange chromatography [4]. Their separation and characterization is possible after appropriate derivatization by glass capillary gas chromatography—mass spectrometry (GC—MS) [5, 6].

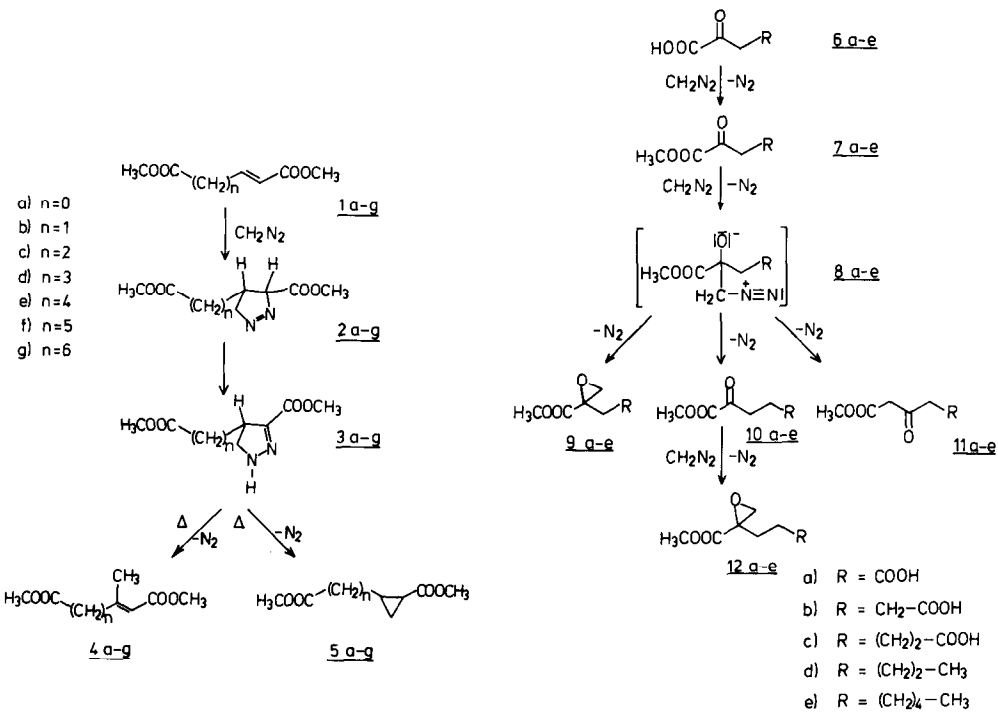
In most cases the acids are transformed into their trimethylsilylated derivatives, which show excellent gas chromatographic properties [7, 8]. Unfortunately, trimethylsilyl esters of acids are rather sensitive to hydrolysis [9]. Therefore any further chromatographic separation step such as liquid chromatography or thin-layer chromatography [10], necessary for the detection of trace compounds, is excluded.

In these cases, methylation of the acid fraction is preferred. Usually this transformation is achieved by treatment with ethereal diazomethane solution. Unfortunately diazomethane not only attacks the acidic hydrogens of acids,

phenols or enols as desired [11], but also adds to the double bonds of α,β-unsaturated esters (Scheme 1, 1a–g) [12] producing Δ^1-pyrazolines (2a–g) which rearrange to Δ^2-pyrazolines (3a–g) [13]. Δ^2-Pyrazolines may decompose in the injector of a gas chromatograph, sometimes producing several thermal reaction products [14] (Scheme 1).

Analogous to the α,β-unsaturated acids, α-oxo acids (Scheme 2, 6a–e) react with diazomethane not only by esterification. The expected methylates (7a–e) are only minor reaction products, because they react further to produce oxiranes (9a–e) [15] by addition of CH_2N_2 to the carbonyl function. This reaction occurs via the zwitterion (8a–e). It can be expected — analogous to the reaction of ketones with diazomethane [11] — that the zwitterion (8a–e) is able to stabilize itself either by elimination of N_2 and ring closure to form the oxirane ring, or by migration of one of the substituents to give the homologous methyl esters (10a–e) (Scheme 2), although migration products or derivatives thereof (12a–e) are not known from the literature [15].

It is rather difficult to recognize undesired reaction products as artefacts without knowledge of their retention indices and mass spectra. We recently reinvestigated the reaction of α,β-unsaturated acids and α-oxo acids with diazomethane to obtain information on the gas chromatographic behaviour and mass spectra of such degradation products to ensure their detection as artefacts in acid fractions of biological fluids and to allow the recognition of the original compounds. We also investigated whether a short reaction time with diazomethane might avoid undesired side-reactions.



Scheme 1. Formation of artefacts from α,β-unsaturated esters.

Scheme 2. Reaction of α-oxo acids with diazomethane.

EXPERIMENTAL

Instruments

A Carlo Erba Fractovap 4160 gas chromatograph was used under the following conditions: hydrogen flow-rate, 2 ml/min; 25-m thin-film glass capillary coated with OV-101; injector temperature, 275° C; oven temperature, 80–280° C; temperature programme, 5 min isothermal, then 2° C/min; flame-ionization detection (FID).

When a Carlo Erba Fractovap 2400T was used for preparative GC, the conditions were: injector temperature, 275° C; oven temperature, 80—260° C; temperature programme, 4° C/min; FID. Column: 1.5 m × 6 mm I.D. filled with 3% OV-17 on Chromosorb W AW DMCS.

For mass spectrometry, a Varian MAT 312 mass spectrometer was used with the following conditions: electron-impact (EI) ion source; electron energy, 70 eV; registration of the total ion current signal at 20 eV. The mass spectrometer was combined with a Varian gas chromatograph 3700, equipped with a 25-m thin-film glass capillary column coated with OV-101; the temperature programme was 5 min isothermal, then 2° C/min; helium flow-rate was 2 ml/min. The instrument was combined with a MAT 200 data system, using a PDP 11/34 computer.

^{1}H-NMR (proton nuclear magnetic resonance) measurements were carried out with a Bruker WM 250 instrument.

Materials

Fumaric acid, maleic acid, laevulinic acid and triphenylphosphine were obtained from E. Merck (Darmstadt, F.R.G.). Cyclopentanone, cyclohexanone, cyclopheptanone, cyclooctanone, glutaconic acid, 2-bromoacetic acid methyl ester, citraconic acid, mesaconic acid, 3-methylglutaconic acid dimethyl ester, 2-oxoglutaric acid, itaconic acid and 3-chloroperbenzoic acid were purchased from EGA-Chemie (Steinheim, F.R.G.). 2-Oxoadipinic acid and 2-oxohexanoic acid (sodium salt) were obtained from Sigma (Munich, F.R.G.) and 2-oxo-octanoic acid was supplied by Fluka (Neu-Ulm, F.R.G.).

Derivatization of α,β-unsaturated dioic acids and of 2-oxo acids

The conversion of an α,β-unsaturated dicarboxylic acid into its dimethyl ester by use of diazomethane is a fast reaction compared to the formation of pyrazolines. Therefore, with regard to the cyclo-addition of CH_2N_2 to α,β-unsaturated acids, it is irrelevant whether the carboxylic acid or the corresponding methyl ester is used.

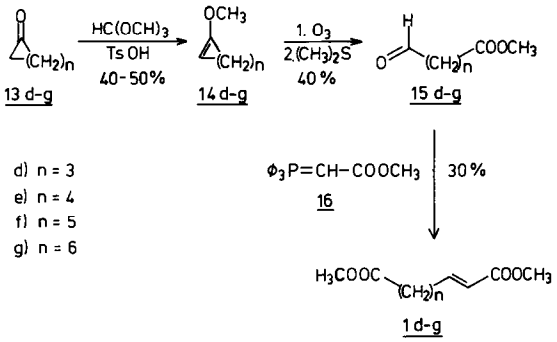
To 1 ml of an ethereal solution containing approximately 1 mg of α,β-unsaturated dioic acid or the corresponding dimethyl ester, or to 1 mg of 2-oxo acid, dissolved in 3 ml of methanol, ethereal diazomethane solution (5%) was added until the solution remained yellow. The excess diazomethane was evaporated by a nitrogen stream. The residue was evaporated to dryness in vacuo and dissolved in 100 μl of ethyl acetate. A 1-μl sample of this solution was injected into the gas chromatograph.

SYNTHESIS OF REFERENCE COMPOUNDS

α,β-Unsaturated dimethyl esters (1a–g)

1c was obtained by hydrolysis of 1,4-dicyanobutene-1 [16] and methylation of the resulting 2-hexenedioic acid by the method of Clinton and Laskowski [17].

The other α,β-unsaturated acids were prepared according to the synthetic approach of Gerlach et al. [18] (Scheme 3) starting from a cycloalkanone (Scheme 3, 13d–g) which was transformed to its methyl enol ether [14d–g). This was subjected to ozonolysis. The resulting ω-oxo ester (15d–g) (0.01 mol) was dissolved in 50 ml of toluene and a slight excess (molar ratio 1:1.1) of β-carbomethoxymethylenetriphenylphosphorane (16) [19] was added. After refluxing for 16 h, the deep yellow solution was cooled to room temperature and brought nearly to dryness in vacuo. Then 20 ml of *n*-hexane were added to the residue and the mixture was stirred at room temperature for 30 min. After filtration, the solvent was evaporated and the residue purified by vacuum destillation.

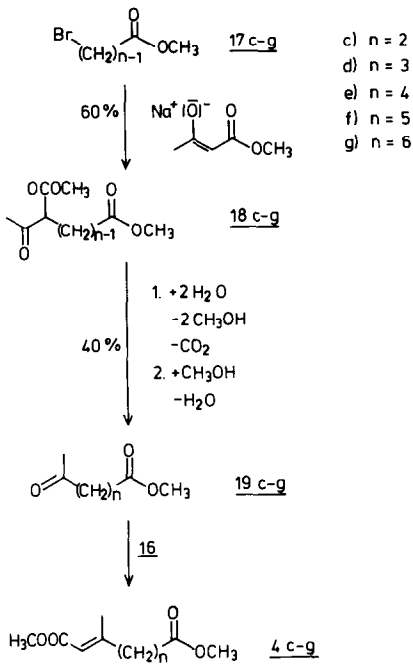


Scheme 3. Synthesis of α,β-unsaturated dimethyl esters.

Synthesis of 3-methylalkenedioic acid dimethyl esters (4c–g) (Scheme 4)

A solution of 0.05 mol of sodium hydride in 100 ml of dimethylformamide-toluene (1:1, v/v) was prepared at 0° C. To this solution 0.05 mol of methyl acetoacetate was slowly added within 20 min. Methyl ω-bromocarboxylate (Scheme 4, 17c–g) was added to the light yellow solution within 10 min. After refluxing for 5 h, the reaction mixture was cooled to room temperature. Then 10 ml of methanol were added. The mixture was stirred for a further 10 min. After addition of 50 ml of water, the mixture was extracted three times each with 50 ml of ether. The combined organic phases were dried over sodium sulphate; the solvent was evaporated and the residue purified by vacuum distillation. The resulting 2-acetylcarboxylates (18c–g) were heated with 30 ml of water to 230° C during 10 h [20]. This Meerwein hydrolysis gave the crude oxocarboxylic acids which were converted to the methyl esters (19c–g) by the method of Clinton and Laskowski [17].

These oxo esters (19c–g) were transformed into the 3-methylalkene dimethyl esters (4c–g) using sodium ylide (16) as described above, except that xylene was used instead of toluene to increase the reaction temperature. The resulting solution of 3-methylalkenedioic acid dimethyl ester (4c–g) was con-



Scheme 4. Synthesis of 3-methylalkenedioic acid dimethyl esters.

centrated to a volume of 5 ml; 50-μl portions were injected into the preparative gas chromatograph. *Z*- and *E*-isomers of the 3-methylalkene diesters (4c–g) were separated from each other, from the solvent and from impurities. The retention times, mass spectral and ^{1}H-NMR data are listed in Table I.

Δ^2-Pyrazolines (3a)

3,4-Dicarbomethoxy-Δ^2-pyrazoline (3a) was prepared according to the description of Roper and Ma [21]. 3-Carbomethoxy-Δ^2-pyrazoline-4-acetic acid methyl ester (3b) was prepared by the method of Birkhofer and Feldmann [22]. The mass spectral and NMR data are listed in Table I.

Cyclopropane derivatives (5a, 5b)

These compounds were synthesized from the methyl esters of acrylic acid, vinylacetic acid and allylacetic acid by addition of carbomethoxycarbene to the C=C double bond [23]. The carbon was generated from methyl diazoacetate by use of Phillips catalyst [24], using argon as inert gas. A 0.5-g amount of methylacrylate (methylvinylacetate or methylallylacetate, respectively) was dissolved in 10 ml of methylene chloride and immediately added to a suspension of 20 g of Phillips catalyst in 50 ml of methylene chloride. The reaction mixture was stirred at room temperature for 15 h. Then the solution was decanted and the catalyst was extracted with 100 ml of methylene chloride. The organic solutions were combined and concentrated to a volume of 1 ml. Then 2 ml of potassium hydroxide solution (10%) were added. After stirring for 10 h at 90°C, the mixture was cooled to room temperature. Then the reaction mixture was extracted twice with 2 ml of diethyl ether and the

TABLE I

SPECTROSCOPIC DATA OF THE SYNTHETIC 3-METHYLALKENEDIOIC ACID DIMETHYL ESTERS

RT = retention time (min), when a preparative gas chromatograph was used.

Compound	RT	RI	MS	NMR
3a		1506	186 (M^+, 2), 153 (4), 127 (42), 95 (100), 68 (10), 59 (30), 56 (9), 42 (15), 39 (10), 28 (12), 15 (50)	3.77 (s), 3.85 (s), 3.94 (dd, J_1 = 9.6 Hz, J_2 = 11.03 Hz), 4.10 (dd, J_1 = 9.6 Hz, J_2 = 11.03 Hz), 6.27 (s)
3b		1598	200 (M^+, 1), 185 (4), 169 (8), 141 (20), 140 (25), 139 (15), 127 (23), 113 (11), 112 (15), 109 (16), 108 (9), 95 (100), 81 (17), 74 (9), 59 (35), 43 (23), 42 (15), 39 (17), 28 (28), 15 (66)	2.5 (dd, J_1 = 10 Hz, J_2 = 16 Hz), 2.9 (dd, J_1 = 3 Hz, J_2 = 16 Hz), 3.40—3.55 (m), 3.58—3.70 (m), 3.70 (s), 3.82—3.91 (m), 3.84 (s), 6.35 (s)
3c		1720	214 (M^+, 1), 199 (5), 186 (17), 167 (24), 155 (48), 154 (13), 151 (20), 148 (10), 139 (31), 127 (13), 123 (22), 122 (26), 109 (24), 95 (100), 85 (10), 81 (36), 67 (18), 59 (47), 55 (25), 42 (24), 41 (26), 39 (29),	
3d		1830	228 (M^+, 3), 213 (3), 195 (16), 181 (18), 169 (54), 163 (15), 140 (16), 137 (26), 109 (45), 95 (100), 82 (26), 81 (40), 74 (16), 59 (57), 55 (35), 41 (42), 39 (29), 28 (41), 15 (87)	
3e		1923	242 (M^+, 4), 227 (4), 209 (19), 195 (14), 183 (33), 151 (15), 149 (14), 127 (18), 123 (22), 109 (18), 95 (100), 82 (41), 81 (25), 59 (48), 55 (32), 42 (28), 41 (27), 39 (21), 28 (37), 15 (63)	
4a, *Z*-isomer		1045	158 (M^+, 3), 127 (100), 126 (17), 99 (24), 98 (4), 68 (16), 59 (44), 39 (29), 29 (19), 15 (40)	1.98 (d, J = 1.6 Hz), 3.69 (s), 3.71 (s), 5.87 (q)
4a, *E*-isomer		1075	158 (M^+, 2), 127 (61), 126 (100), 99 (26), 98 (29), 68 (42), 59 (63), 39 (56), 29 (25), 15 (70)	2.30 (d, J = 1.6 Hz), 3.71 (s), 3.78 (s), 6.80 (q)

TABLE I (continued)

Compound	RT	RI	MS	NMR
4b, *Z*-isomer	17	1162	172 (M^+, 1), 141 (53), 140 (100), 113 (31), 112 (94), 109 (30), 108 (8), 97 (39), 85 (9), 82 (19), 81 (31), 59 (38), 55 (31) 53 (49), 41 (20), 39 (37), 27 (28), 15 (63)	1.98 (d), 3.69 (s), 3.71 (s), 3.75 (s), 5.86 (q)
4b, *E*-isomer	19	1198	172 (M^+, 2), 141 (32), 140 (100), 113 (44), 112 (87), 109 (19), 108 (21), 97 (26), 85 (10), 82 (20), 81 (28), 59 (42), 55 (36), 53 (38), 41 (18), 39 (37), 27 (28), 15 (64)	2.30 (d, J = 1.6 Hz), 3.71 (s), 3.78 (s), 5.80 (q, J = 1.6 Hz)
4c, *Z*-isomer	19	1278	186 (M^+, 2), 155 (43), 154 (100), 127 (31), 126 (37), 123 (17), 122 (51), 111 (19), 95 (94), 94 (40), 85 (42), 67 (62), 59 (32), 55 (22), 53 (16), 41 (43), 39 (38), 27 (24), 15 (60)	1.91 (d, J = 1.4 Hz), 2.47—2.52 (m), 2.88—2.95 (m), 368 (s), 5.72 (q, J = 1.4 Hz)
4c, *E*-isomer	21	1287	186 (M^+, 2), 155 (38), 154 (96), 127 (29), 126 (42), 123 (22), 122 (55), 111 (21), 95 (100), 94 (41), 85 (44), 67 (56), 59 (41), 55 (25), 53 (20), 41 (41), 39 (40), 27 (24), 15 (62)	2.18 (d, J = 1.4 Hz), 3.69 (s), 4.80—4.52 (m), 5.68 (q, J = 1.4 Hz)
4d, *Z*-isomer	22	1373	169 (44), 168 (96), 140 (22) 137 (28), 136 (57), 127 (19), 125 (20), 109 (100), 108 (45), 99 (22), 95 (77), 82 (26), 81 (65), 74 (17), 69 (18), 67 (48), 59 (46), 55 (41), 53 (31), 43 (32), 41 (50), 39 (52), 27 (23), 15 (71)	1.78—185 (m), 1.90 (d, J = 1.3 Hz), 2.37 (t, J = 7.6 Hz), 2.64—2.70 (m), 3.67 (s), 5.67 (q, J = 1.3 Hz)
4d, *E*-isomer		1420	169 (33), 168 (68), 140 (20), 137 (29), 136 (51), 127 (17), 125 (20), 109 (100), 108 (41), 99 (27), 95 (80), 82 (41), 81 (72), 74 (18), 69 (16), 67 (38), 59 (45), 55 (57), 53 (31), 43 (38), 41 (43), 39 (47), 29 (16), 27 (32), 15 (63)	1.78—1.90 (m), 2.16 (d, J = 1.2 Hz), 2.16—2.18 (m), 2.90—2.35 (m), 3.68 (s), 3.69 (s), 5.68 (q, J = 1.2 Hz)
4e, *Z*-isomer	24	1479	183 (30), 182 (49), 155 (13), 154 (23), 151 (18), 150 (62), 123 (25), 122 (51), 109 (26), 108 (28), 96 (23), 95 (100),	1.45—1.48 (m), 167—1.75 (m), 1.89 (d, J = 1.5 Hz), 2.30—2.40 (m), 2.60—2.67 (m), 3.67 (s), 5.68 (br)

(Continued on p. 250)

TABLE I (continued)

Compound	RT	RI	MS	NMR
			82 (25), 81 (32), 67 (36), 59 (38), 55 (31), 53 (19), 43 (18), 41 (37), 39 (33), 29 (38), 27 (19), 15 (56)	
4e, *E*-isomer	26	1530	183 (18), 182 (29), 154 (20), 155 (9), 151 (18), 150 (53), 123 (18), 122 (43), 109 (22), 108 (25), 96 (21), 95 (100), 82 (48), 81 (35), 67 (31), 59 (48), 55 (38), 53 (22), 43 (17), 41 (40), 39 (29), 29 (17), 27 (20), 15 (52)	1.23—1.31 (m), 1.45—1.70 (m), 2.12—2.20 (m), 2.15 (d, J = 1.5 Hz), 2.30—2.40 (m), 3.67 (s), 3.68 (s), 5.66 (br)
4f, *Z*-isomer	27	1571	197 (22), 196 (34), 168 (10), 164 (39), 137 (28), 136 (43), 114 (13), 109 (40), 108 (25), 96 (37), 95 (100), 83 (20), 82 (35), 81 (19), 67 (35), 59 (37), 55 (48), 43 (26), 41 (49), 39 (30), 29 (24), 27 (24), 15 (45)	1.33—1.56 (m), 1.60—1.70 (m), 1.88 (d, J = 1.3 Hz), 2.32 (t, J = 7.5 Hz), 2.62 (t, J = 7.5 Hz) 3.67 (s), 5.60 (br)
4f, *E*-isomer	28	1621	197 (23), 196 (36), 169 (14), 164 (40), 137 (30), 136 (39), 114 (12), 109 (41), 108 (25), 96 (34), 95 (100), 83 (20), 82 (71), 81 (20), 67 (34), 59 (46), 55 (49), 53 (19), 43 (18), 41 (45), 39 (32), 29 (20), 27 (22), 15 (53)	1.25—1.70 (m), 2.10—2.20 (m), 2.14 (d, J = 1.3 Hz), 2.32 (t, J = 7.6 Hz), 3.67 (s), 3.68 (s), 5.66 (q, J = 1.3 Hz)
4g, *Z*-isomer	29	1672	211 (16), 210 (22), 182 (15), 178 (18), 151 (22), 150 (33), 133 (21), 114 (18), 109 (38), 108 (27), 96 (33), 95 (100), 82 (37), 81 (21), 74 (17), 69 (32), 67 (37), 59 (34), 55 (40), 41 (53), 39 (21), 29 (16), 27 (15), 43 (16)	1.25 —1.69 (m), 1.88 (d, J = 1.3 Hz), 2.31 (t, J = 7.4 Hz), 2.61 (t, J = 7.5 Hz), 3.67 (s), 3.68 (s), 5.65 (br)
4g, *E*-isomer		1730	211 (17), 210 (19), 182 (13), 178 (17), 151 (23), 150 (31), 133 (22), 114 (23), 109 (42), 108 (32), 96 (34), 95 (89), 82 (100), 81 (28), 74 (28), 69 (40), 67 (39), 59 (50), 55 (63), 43 (23), 41 (66), 29 (25), 27 (29), 15 (54)	1.22—1.68 (m), 2.10—2.18 (m), 2.14 (d, J = 1.3 Hz), 2.25—2.35 (m), 3.67 (s), 3.69 (s), 5.66 (br)
5a, *Z*-isomer	16	1101	157 (M^+— 1, 2), 127 (100), 126 (15), 99 (38), 98 (33), 95 (7), 71 (20), 59 (37), 55 (14), 41 (28), 39 (31), 29 (10), 27 (15), 15 (58)	1.21—1.30 (m), 1.65—1.72 (m), 2.08 (dd, J_1 = 6.6 Hz, J_2 = 8.5 Hz), 3.70 (s)

TABLE I (continued)

Compound	RT	RI	MS	NMR
5a, *E*-isomer	14	1090	157 (M^+-1, 2), 127 (100), 126 (41), 99 (60), 98 (85), 95 (13), 85 (5), 83 (8), 71 (14), 59 (57), 55 (22), 41 (33), 39 (54), 29 (20), 27 (22), 15 (85)	1.42 –1.45 (m), 2.15–2.20 (m), 3.70 (s)
5b, *Z*-isomer	20	1175	141 (17), 140 (21), 113 (16), 112 (14), 109 (57), 108 (46), 99 (53), 98 (64), 81 (48), 71 (32), 59 (63), 55 (29), 53 (41), 41 (34), 39 (27), 27 (38), 15 (100)	0.97–1.02 (m), 1.10–1.19 (m), 1.55–1.60 (m), 2.30–2.54 (m), 3.68 (s), 3.69 (s)
5b, *E*-isomer	22	1190	141 (15), 140 (19), 113 (29), 112 (17), 109 (24), 108 (36), 99 (67), 98 (58), 81 (42), 71 (48), 59 (80), 55 (34), 53 (33), 27 (41), 15 (100)	0.80–1.86 (m), 1.23–1.31 (m), 1.46–1.50 (m), 1.62–1.80 (m), 3.70 (s), 3.69 (s)

organic solution was discarded. The aqueous phase was brought to pH 1 by adding concentrated hydrochloric acid and then extracted with 4 ml of diethyl ether. After methylation of the resulting organic solution by diazomethane, the ether was removed by a stream of nitrogen, and the residue redissolved in 2 ml of methanol; 50 μl of this solution were injected into the preparative gas chromatograph. Retention times, mass spectral and ^{1}H-NMR data are given in Table I.

2-(Carbomethoxy)-oxirane-acetic acid methyl ester (9a)

Seven grams (0.044 mol) of itaconic acid dimethyl ester, prepared from itaconic acid with methanol [17], were dissolved in 40 ml of methylene chloride; 9 g (85%) of 3-chloroperbenzoic acid, dissolved in 100 ml of methylene chloride, were slowly added with stirring and raising the temperature to 36° C. The solution was stirred a further 12 h at room temperature. Most of the 3-chlorobenzoic acid precipitated during this procedure. It was filtered off and the filtrate was washed three times each with 100 ml of 2 *M* sodium bicarbonate.

After evaporation of the solvent, 2 g of the residue were dissolved in 10 ml of cyclohexane and chromatographed on 180 g of silica gel (Fluka, 60) with cyclohexane–ethyl acetate (2:1) as solvent (^{1}H-NMR [25]).

2-(Carbomethoxy)-oxirane-propionic acid methyl ester (9b)

2-(Carbomethoxy)-oxirane-propionic acid methyl ester (9b) was prepared according to the procedure described for the synthesis of 9a, by treatment of 2-methyleneglutaric acid dimethyl ester [26] with 3-chloroperbenzoic acid (^{1}H-NMR [15]).

RESULTS

Reaction of α,β-unsaturated acids with diazomethane

The rate of production of pyrazolines by treatment of α,β-unsaturated

alkenedioic acids or their dimethyl esters (1a—g) is strongly dependent on the molecular structure: with maleic and fumaric acid Δ^2-pyrazoline formation (Scheme 1) is quantitatively complete within 30 sec. Higher homologues react much more slowly. If the reaction is stopped after 1 min, the production of pyrazolines can be nearly avoided. If a Δ^2-pyrazoline (3) is subjected to gas chromatography it does not form a sharp peak but a broad signal if OV-101 is used as stationary phase. Plotting the molecular weights of the homologues against their retention indices (RI) results in a straight line.

If the temperature of the injector is kept at about 280° C no significant decomposition of the pyrazolines (3a—g) is observed, but if the injector is heated to 320° C or more, the pyrazolines (3a—g) are partly decomposed producing the

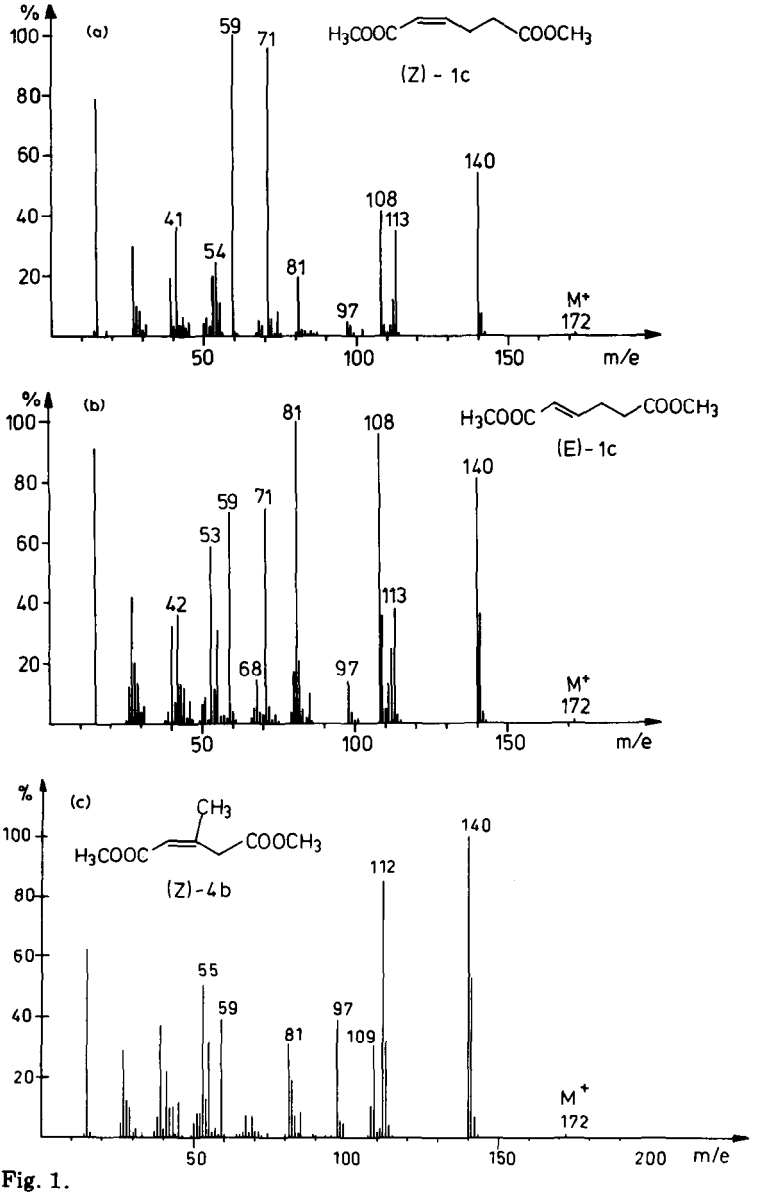


Fig. 1.

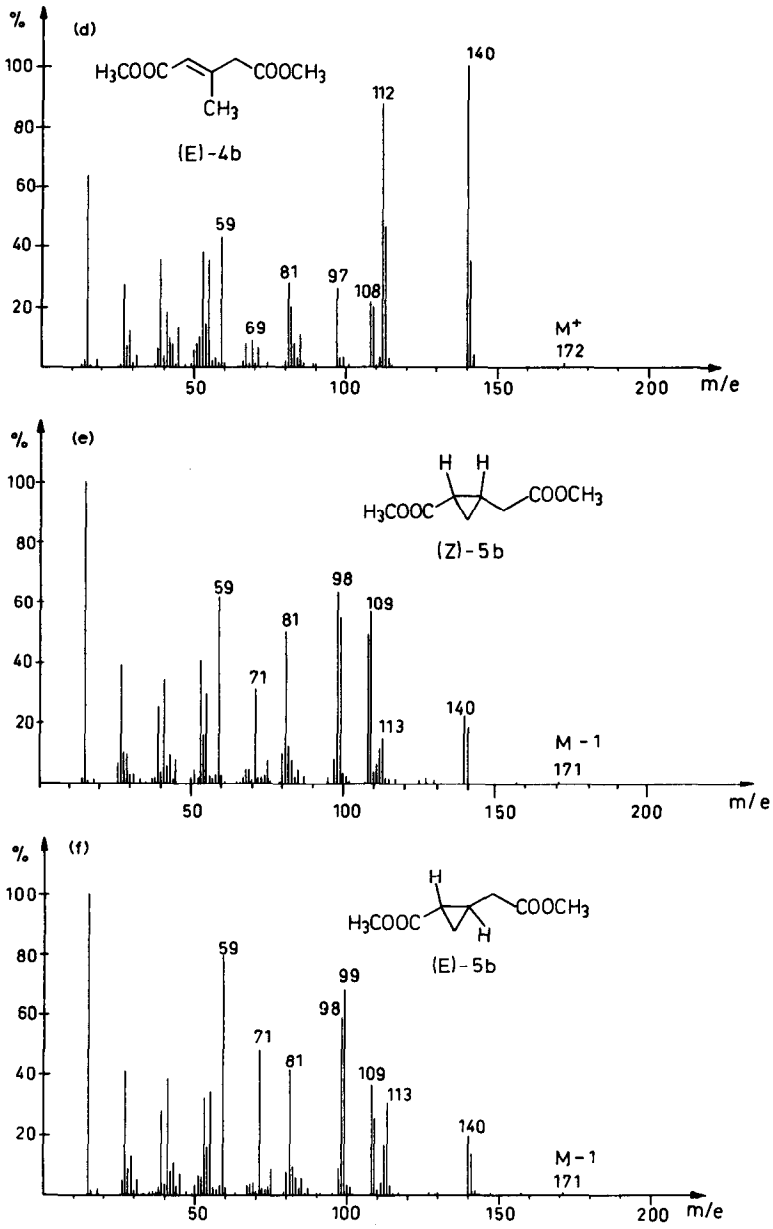


Fig. 1. Mass spectra of the methyl esters of hexenedioic acid (1c), 3-methylglutaconic acid (4b) and 1-carboxycyclopropane-2-acetic acid (5b) (both *Z*- and *E*-isomers).

methyl esters of 3-methylalkenedioic acids (4a—g) (*Z*- and *E*-isomers) as well as the corresponding cycloalkane derivatives (5a—g) (Scheme 1). The Δ^2-pyrazolines (3a—g) can be characterized not only by their RI values but also by their characteristic mass spectral fragmentation reactions. The most characteristic fragment in the mass spectrum of 3 is *m/z* 95. This ion is probably produced via the ion of *m/z* 127 by elimination of CH_3OH.

The *Z*- and *E*-isomers of 4 are well separated by gas chromatography (Table

I). Both isomers, and also their straight chain isomers, exhibit the same fragment ions with ions of $M^+ - 31$, $M^+ - 32$, $M^+ - 59$, $M^+ - 64$ and $M^+ - 73$ in their mass spectra, only differences in intensity being observed. Therefore identification requires a careful comparison with authentic compounds. Even the *Z*- and *E*-isomers can be distinguished if mass spectra and RI values are carefully compared.

Equally difficult is the differentiation between the isomeric straight-chain alkenedioic acid esters and cyclopropanedioic acid esters (5a—g) by comparison of their mass spectra alone. The mass spectra of the cyclopropane dioic acid methyl esters (5a—g) show the same main fragmentations as the mass spectra of the corresponding 3-methylalkenedioic acid dimethyl esters (4a—g).

The difficulties encountered in the identification of α,β-unsaturated esters (4a—g) and cyclopropanedioic acid dimethyl esters (5a—g) as well as *Z*- and *E*-isomers of 3-methylalkenedioic dimethyl esters (4a—g) by MS alone are demonstrated in Fig. 1.

Reactions of α-oxo acids with diazomethane

The GC analysis of the reaction products obtained from 2-oxoglutaric acid (6b) with diazomethane (Fig. 2) yielded as main product 2-(carbomethoxy)-oxirane-propionic acid methyl ester (9b) (88%); 2-oxoglutaric acid dimethyl

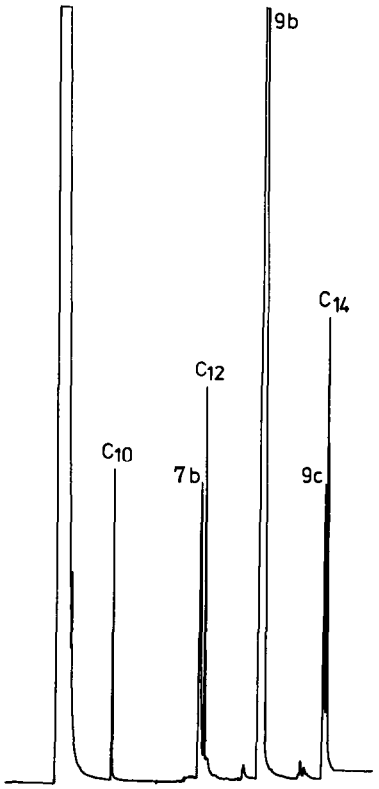


Fig. 2. Glass capillary gas chromatogram of the products formed by the reaction of 2-oxoglutaric acid with diazomethane.

TABLE II

REACTION PRODUCTS OF 2-OXO ACIDS WITH DIAZOMETHANE

Compound	RI	MS	%
9a	1180	174 (M^+, 0.5), 159 (1), 143 (11), 142 (6), 116 (5), 115 (100), 110 (18), 101 (10), 87 (8), 83 (17), 82 (2), 74 (15), 69 (3), 59 (34), 57 (35), 55 (9), 45 (48)	52
7b	1185	174 (M^+, 1), 143 (5), 142 (2), 116 (8), 115 (100), 87 (18), 59 (56), 55 (63), 45 (10), 43 (7)	5
9b	1292	188 (M^+, 0.5), 173 (2), 157 (4), 156 (3), 141 (11), 129 (34), 128 (20), 125 (30), 116 (6), 115 (98), 113 (10), 111 (8), 101 (12), 97 (18), 96 (9), 87 (18), 83 (8), 71 (7), 69 (21), 68 (7), 59 (54), 55 (94), 53 (6), 45 (100), 43 (16), 42 (20), 41 (21)	88
12b = 9c	1390	202 (M^+, 1), 172 (3), 171 (4), 170 (1), 155 (9), 152 (12), 143 (34), 142 (26), 139 (8), 129 (19), 128 (20), 124 (10), 115 (38), 111 (20), 101 (21), 97 (18), 83 (37), 81 (17), 71 (23), 69 (19), 59 (90), 55 (100), 45 (98), 43 (38), 42 (40), 41 (42)	5
7c	1281	188 (M^+, 1), 157 (10), 129 (78),101 (48), 97 (12), 59 (100), 55 (60), 42 (26), 41 (22)	4
12c	1497	216 (M^+, 4), 185 (6), 169 (16), 167 (10), 157 (39), 153 (11), 134 (36), 125 (27), 115 (64), 111 (43), 106 (20), 97 (27), 85 (30), 83 (50), 81 (24), 79 (39), 74 (22), 69 (20), 68 (28), 67 (30), 59 (90), 55 (100), 45 (97), 43 (88), 41 (63)	2
7d	1010	144 (M^+, 6), 102 (1), 85 (82), 59 (10), 57 (100), 55 (9), 41 (52)	2
9d	1083	158 (M^+, 1), 141 (2), 129 (4), 128 (1), 127 (1), 115 (44), 99 (10), 85 (38), 83 (12), 81 (14), 69 (8), 59 (18), 57 (33), 55 (18), 45 (49), 43 (100), 41 (34)	89
12d	1192	172 (M^+, 1), 143 (4), 141 (1), 129 (3), 125 (11), 115 (32), 99 (17), 95 (18), 87 (11), 85 (18), 83 (6), 81 (5), 71 (17), 69 (12), 59 (19), 55 (30), 45 (37), 43 (100), 41 (34)	9
7e	1195	172 (M^+, 1), 113 (50), 85 (20), 69 (5), 59 (7), 57 (14), 55 (10), 43 (100), 41 (21)	2
9e	1294	186 (M^+, 0.5), 155 (1), 153 (2), 125 (11), 117 (11), 115 (36), 113 (15), 109 (19), 101 (9), 85 (30), 69 (24), 67 (12), 59 (16), 55 (32), 45 (37), 43 (100), 41 (44)	90
12e	1392	200 (M^+, 2), 179 (1), 169 (1), 168 (1), 153 (4), 141 (6), 127 (8), 125 (15), 123 (18), 117 (19), 115 (43), 100 (13), 85 (38), 83 (12), 81 (11), 71 (11), 69 (15), 67 (20), 59 (22), 57 (43), 55 (61), 45 (42), 43 (100), 41 (56)	8

ester (7b) was produced only in 5% yield. A third peak in the gas chromatogram showed in its mass spectrum a parent ion peak at *m/z* 202 and key ions at mass *m/z* 45, *m/z* 55, *m/z* 59, *m/z* 101, and *m/z* 115, which suggested that its structure differed from the oxirane (*m/z* 188) (9b) by the presence of an additional methylene group. This assumption was confirmed by reacting 2-oxoglutaric acid (6b) with deuterodiazomethane [27], demonstrating the introduction of four C^2H_2 groups into the original molecule.

The ^{1}H-NMR spectrum of the isolated unknown compound of 202 molecular weight, revealed a pair of doublets at δ = 3.05 ppm and δ = 2.81 ppm (J = 6 Hz), assigned to an AB proton for oxirane ring protons, suggesting structure 9c for the compound.

To confirm this assumption 2-oxoadipinic acid (6c) was treated with diazomethane. The resulting main product 2-(carbomethoxy)-oxirane-butyric acid methyl ester (9c) showed the same mass spectrum and RI value as the by-product of the reaction of 2-oxoglutaric acid (6b) with diazomethane. Obviously, by migration of the zwitterion (8b), 2-oxoadipic acid methyl ester (7c = 10b) is formed which reacts immediately with diazomethane to give 12b = 9c as indicated in Scheme 2. The corresponding oxirane of 11b could not be detected.

All investigated compounds [2-oxoglutaric acid (6b), 2-oxoadipic acid (6c), 2-oxohexanoic acid (6d) and 2-oxooctanoic acid (6e)] showed an analogous behaviour on treatment with diazomethane (Table II).

Solvent effects

In general, the weak acid methanol increases the ratio of produced oxiranes [28] by increasing the polarization of diazomethane forming hydrogen bonds. Therefore we tried to see if the oxirane production could be avoided or at least reduced by the use of less polar solvents. The results of these experiments (Table III) demonstrate only negligible effects.

TABLE III

SOLVENT EFFECTS

Solvent	2-Oxoglutaric acid dimethyl ester (7b) (%)	2-(Carbomethoxy)-oxirane-propionic acid methyl ester (9b) (%)	2-(Carbomethoxy)-oxirane-butyric acid methyl ester (9c) (%)
Cyclohexane	48.2	51.5	0.3
Without solvent	59	41	—
Ether	55	45	—
Benzene	47.3	52.1	—
Ethyl acetate	53	47	—
Methanol	5	88	5

Reaction time

Reduction of the reaction time between acid and diazomethane from 10 min to 1 min (solvent methanol), increased the rate of dimethyl ester produced from 2-oxoglutaric acid (6b) from 5% to 59% and reduced the rate of oxirane

dimethyl ester (9b) production from 88% to 41%. Thus the production of oxirane can not be avoided but can be considerably reduced.

Mass spectra

In all oxiranes obtained from α-oxo methylates an intense ion is observed at *m/z* 115 ($C_5H_7O_3^+$) (Fig. 3) formed by β-cleavage from the molecule, a marked contrast to acyclic ethers in which α-cleavage is the predominant fission reaction [29]. The ion *m/z* 115 loses CH_3OH and CO to give the ion *m/z* 55 (base peak). Typical further key ions are found at *m/z* 45 ($C_2H_5O^+$), *m/z* 59 ($C_2H_3O_2^+$), *m/z* 83 ($C_4H_3O_2^+$) and *m/z* 101 ($C_4H_5O_3^+$) allowing firm identification of this class of compounds (Scheme 5).

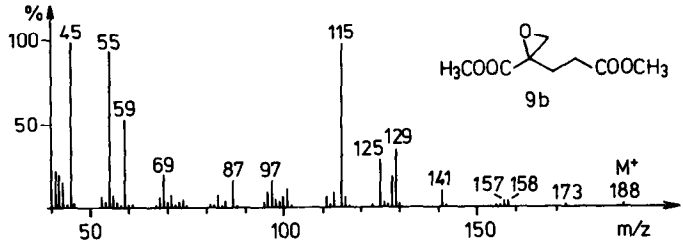
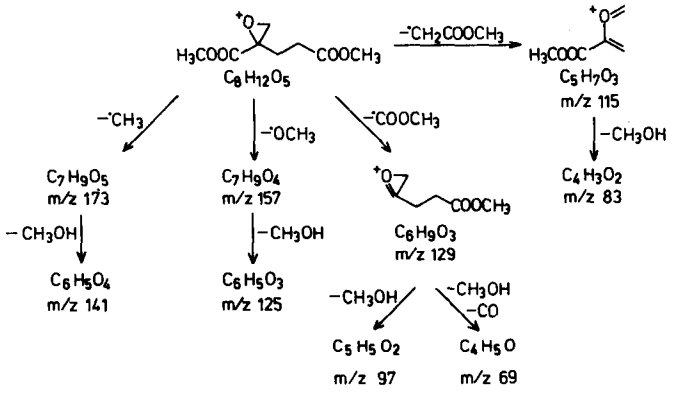


Fig. 3. Mass spectrum of 2-(carbomethoxy)-oxirane-propionic acid methyl ester (9b).



Scheme 5. Fission reactions of 2-(carbomethoxy)-oxirane-propionic acid methyl ester (9b).

Identification of "unknown" compounds in urine

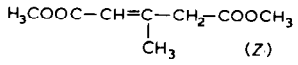
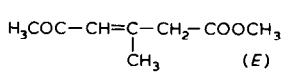
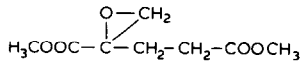
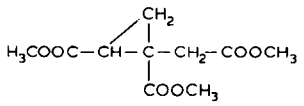
Some time ago our group published a paper [10] on the identification of acidic compounds in urine by glass capillary gas chromatography after treatment with diazomethane. Several of the peaks remained unknown since no comparison material was available. Some of these compounds have now been positively identified as artefacts from the compounds listed in Table IV.

DISCUSSION

It is not possible to stop the reaction of diazomethane with α,β-unsaturated acids or α-oxo acids at the step of the methyl ester, since these compounds

TABLE IV

COMPOUNDS POSITIVELY IDENTIFIED AS ARTEFACTS

Peak number in GC of urine (ref. 10)	RI	Structure	Artefact of
34	1163		
39	1195		
56	1291		2-Oxoglutaric acid
92	1428		Aconitic acid
98	1440	Isomers of 92	Aconitic acid
101	1445	Isomers of 92	Aconitic acid

react further to produce either mainly pyrazolines or oxiranes — even if very short reaction times are used.

The GC retention indices and mass spectral data of those reaction products are presented in this paper. These data are important in recognizing these compounds in complicated mixtures of acids obtained from biological fluids and in identifying the original compounds. Consequently a time-consuming structural elucidation is also avoided.

Due to the lack of key ions, characterization of them and decomposition products of Δ^2-pyrazolines is not possible from the mass spectra alone but requires also the measurement and comparison of RI values.

The accumulated data obtained from the most common α,β-unsaturated acids and α-oxo acids may also enable the detection of similar artefacts, not listed in the tables, by recognition of typical mass spectrometric key ions (for instance, ions of mass *m/z* 45, *m/z* 55, *m/z* 101 and *m/z* 115 for oxirane methyl esters, and ions of mass *m/z* 95 and *m/z* 127 for Δ^2-pyrazolines).

ACKNOWLEDGEMENTS

We thank the Robert Pfleger-Stiftung in Bamberg and Fonds of the Chemischen Industrie for financial support. Thanks are also due to Dr. Josef Reiner for the ^{1}H-NMR measurements and M. Glaessner for running mass spectra. We thank D. Laatsch for providing the glass capillary columns.

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