The aliphatic acetals were prepared by standard methods and were purified by fractional distillation from potassium metal. The dioxolans were prepared by the method of LUCAS AND GUTHRIE[5] and were purified by fractional distillation from potassium metal.

Results and discussion

The relative detector responses to a number of aliphatic and cyclic ethers and aliphatic and cyclic acetals are shown in Table I.

An examination of these results shows that there is, in each homologous series, an increase in relative response with an increase in molecular weight. The increment for each CH_2 group is approximately 22 units of response for aliphatic ethers, 21 units for the methylfurans, 21 units for the aliphatic acetals. These increments are similar to those found for other homologous series[6]. The increment for the dioxolans is only 11 units per CH_2 group.

It is also found that, in the aliphatic ethers and acetals, there is a decrease in relative response to isomeric compounds with an increase in chain branching. This decrease in relative response is similar to those found previously for isomeric alkylbenzenes[3] and for isomeric aliphatic esters[4].

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Separation and identification of amino acids by two-dimensional paper chromatography

The separation and identification of amino acids present in a mixture have been carried out by paper partition chromatography using several different solvent systems[1]. In each solvent system certain amino acids have R_F values that are so similar that it is often difficult to distinguish them. This difficulty has been overcome to a certain extent by subjecting a mixture of amino acids to two-dimensional chromatography, using a different solvent system for each dimension[1–5]. For this purpose, the mixture containing the unknown amino acids is generally applied to a filter paper sheet near one of its corners. After running a solvent through the paper sheet in one direction, it is dried and a second solvent is run through it in a direction at right

angles to the first. The amino acids, thus separated in both directions, are revealed as coloured spots by treating the paper with ninhydrin or some other reagent[1]. The amino acids are identified by comparing their relative positions or R_F values with those of known amino acids developed on separate paper sheets with the same solvents. It has been observed that the relative positions or R_F values of the amino acids for a solvent system are subjected to fluctuation from one chromatogram to another on account of a number of factors[1]. The most convenient and reliable method of identifying amino acids on a chromatogram would then be to compare their positions with those of the known amino acids developed simultaneously on the same paper. This procedure has often been adopted for one-dimensional but not for two-dimensional chromatography. This is due to two main difficulties: first, the number of known amino acids used as references for identification is often quite large. Since the unknown sample is applied close to a corner of the paper sheet, there is hardly any space left to accommodate all the reference amino acids. Secondly, if the reference amino acids move along with the test sample in both directions on the same paper sheet, they would get mixed up with the unknown amino acids of the mixture. On account of this identification would not be possible.

Bearing these difficulties in mind, it has been possible to develop a method by

TABLE I

R_L VALUES OF VARIOUS AMINO ACIDS SUBJECTED TO TWO-DIMENSIONAL CHROMATOGRAPHY

First solvent: *n*-butanol–acetic acid–water (4:1:5, v/v; butanol phase); second solvent: *n*-propanol–diethylamine–water (85:4:15, v/v)

Amino acids	*R_L values (in different chromatograms)**									
	A		*B*		*C*		*D*		*E*	
	sol_1	sol_2	sol_1	sol_2	sol_1	sol_2	sol_1	sol_2	sol_1	sol_2
Norleucine	1.06	0.98	1.06	1.00	1.05	1.00	1.05	0.92	1.04	0.97
Leucine	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Isoleucine	0.94	0.94	0.94	0.97	0.81	0.98	0.92	0.90	0.93	0.93
Phenylalanine	0.87	0.96	0.87	0.98	0.85	0.98	0.86	0.94	0.83	0.98
Norvaline	0.78	0.78	0.77	0.76	0.77	0.81	0.76	0.75	0.74	0.78
Tryptophan	0.65	0.48	0.63	0.47	0.60	0.52	0.64	0.50	0.66	0.54
Methionine	0.64	0.70	0.67	0.66	0.63	0.70	0.65	0.66	0.63	0.70
Tyrosine	0.52	0.57	0.48	0.58	0.47	0.60	0.52	0.54	0.50	0.60
Alanine	0.33	0.40	0.32	0.40	0.30	0.45	0.36	0.40	0.32	0.40
Threonine	0.26	0.76	0.23	0.63	0.23	0.78	0.25	0.73	0.23	0.76
Glutamic acid	0.25	0.06	0.23	0.04	0.22	0.05	0.26	0.07	0.24	0.08
Proline	0.18	0.21	0.18	0.22	0.17	0.22	0.20	0.25	0.19	0.26
Glycine	0.17	0.20	0.16	0.17	0.15	0.16	0.17	0.19	0.17	0.18
Aspartic acid	0.17	0.06	0.15	0.03	0.12	0.04	0.17	0.05	0.17	0.06
Serine	0.16	0.28	0.15	0.25	0.14	0.29	0.16	0.21	0.15	0.29
Citrulline	0.13	0.10	0.13	0.08	0.12	0.10	0.15	0.09	0.13	0.11
Taurine	0.11	0.45	0.10	0.47	0.10	0.51	0.13	0.42	0.13	0.46
Arginine	0.10	0.07	0.08	0.05	0.08	0.06	0.11	0.07	0.10	0.08
Histidine	0.08	0.32	0.07	0.29	0.07	0.32	0.09	0.34	0.07	0.34
Asparagine	0.07	0.12	0.07	0.10	0.07	0.11	0.09	0.13	0.08	0.13
Lysine	0.06	0.11	0.06	0.09	0.05	0.09	0.07	0.10	0.06	0.11
Ornithine	0.05	0.06	0.05	0.05	0.04	0.05	0.06	0.09	0.05	0.10
Cystine	0.02	0.05	0.02	0.04	0.02	0.04	0.03	0.02	0.02	0.05

* R_L value is the ratio of the distance travelled by an amino acid to that travelled by leucine; sol_1 and sol_2 are the first and second solvents respectively.

which the amino acids in a mixture can be separated by two-dimensional chromatography and identified on the basis of reference amino acids run simultaneously on the same paper sheet.

The present note describes the method in which 23 amino acids (Table I) were used as references. The solvent employed for the first dimension was the butanol phase of a mixture of *n*-butanol, acetic acid and water (4:1:5 v/v). For the second dimension, *n*-propanol–diethylamine–water (85:4:15 v/v) was used as the solvent. On a 22 in. × 18 in. sheet of filter paper (Whatman No. 1) (Fig. 1) three parallel lines were drawn at distances of 1 (I), 2 (II), and 7 (EH) inches from one of the longer edges (AD) of the paper. Similarly, four parallel lines were drawn at distances of 7 (IJ), 8 (I′), 9 (II′) and 10 (KL) inches from the shorter edge of the paper. The mixture containing solutions of all the 23 amino acids was applied at the junction (G)

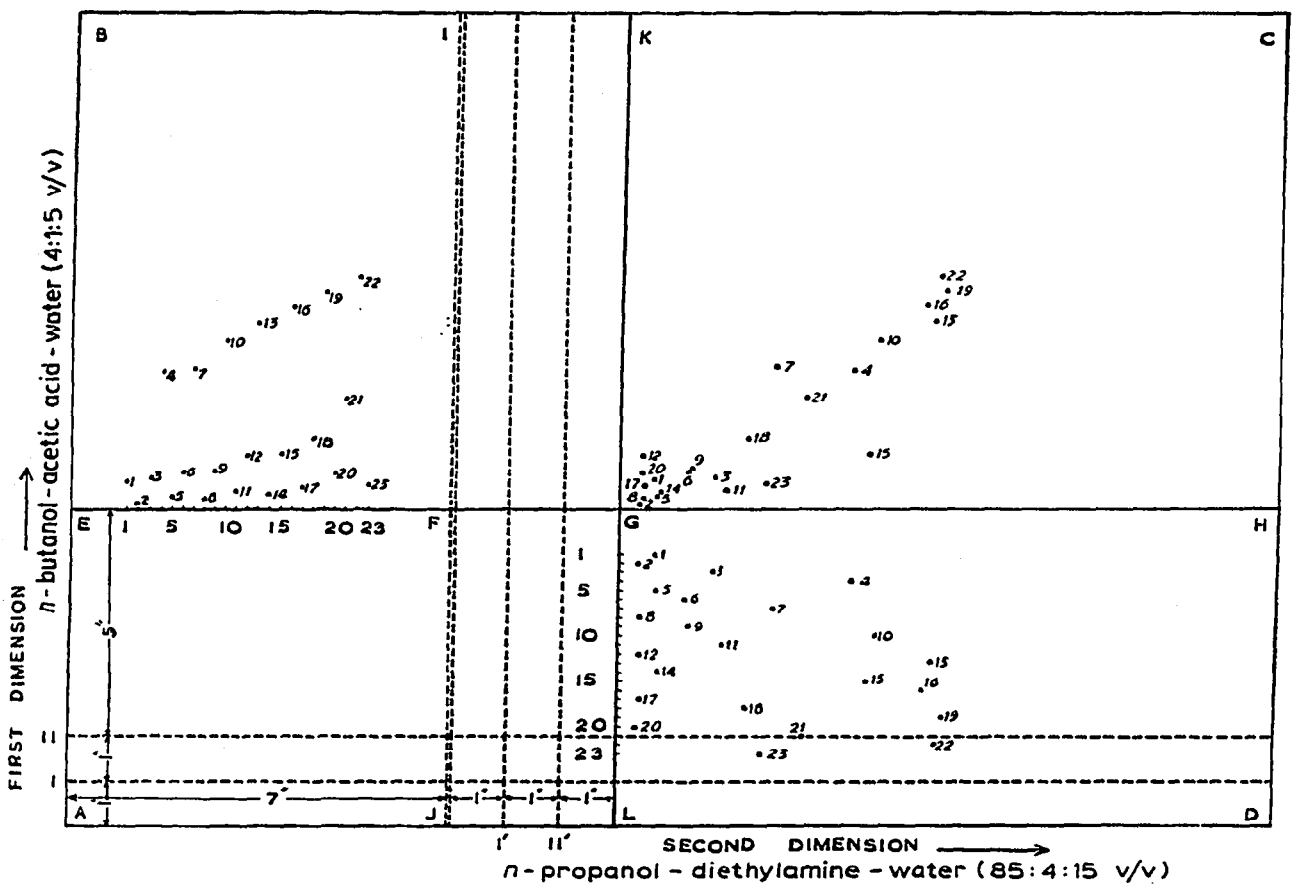

Fig. 1. Diagrammatic representation of a two-dimensional chromatogram of amino acids using *n*-butanol–acetic acid–water (4:1:5 v/v; butanol phase) as the first and *n*-propanol–diethylamine–water (85:4:15 v/v) as the second solvent. ABCD: sheet of Whatman No. 1 filter paper, 22 in. long and 18 in. wide. EH and KL: base lines for the first and second dimensions respectively. G: spot of the mixture of the amino acids. IJ: line along which the paper is cut after the first solvent has been run. I and I′: first fold for the first and second dimensions respectively. II and II′: second fold for the first and second dimensions respectively. EBIF: section of the chromatogram containing reference amino acids for the first dimension. GHDL: section containing reference amino acids for the second dimension. KCHG: section containing amino acids of the mixture. (1) citrulline; (2) cystine; (3) serine; (4) methionine; (5) lysine; (6) glycine; (7) tryptophan; (8) ornithine; (9) proline; (10) norvaline; (11) histidine; (12) glutamic acid; (13) phenylalanine; (14) asparagine; (15) threonine; (16) isoleucine; (17) arginine; (18) alanine; (19) leucine; (20) aspartic acid; (21) tyrosine; (22) norleucine; (23) taurine.

of the lines EH and KL. Care was taken to ensure that the spot of the mixture on the paper did not spread beyond a diameter of 0.2 in. The reference amino acids for the first dimension were spotted along the base line EH on the left of the spot G of the mixture, leaving a gap of at least 4 inches from this point and 1 inch from the free edge (AB) of the sheet. The references were placed 0.2 in. apart, each spot being about 0.1 in. in diameter. The distances over which the amino acids travelled with the first solvent were determined on separate chromatograms. On the basis of this, the references were arranged along the base line EH in such a way that any three adjacently placed amino acids differed markedly from each other in their relative mobility (Fig. 1 sector EBIF). As a result, confluence of the spots of reference amino acids placed very close together on the base line in the developed chromatogram was avoided and their spots could be easily distinguished from each other.

After drying the reference amino acids applied on the base line, the paper-sheet was folded at the lines I and II, and transferred to a large chromatography chamber (26 in. high, 24 in. long, and 12 in. wide). The part of the paper-sheet proximal to the first fold (I) was immersed in the first solvent contained in a glass trough. The rest of the sheet was suspended downwards. The first fold (I) was supported by the edge of the trough and the second fold (II) by a glass rod at a distance of about 1 in. from the trough. The solvent was allowed to run down the paper sheet up to the edge BC. It was then air-dried and the sector ABIJ, containing the reference amino acids, was cut off. On the remaining paper sheet (ICDJ), another set of the same reference amino acids was similarly plotted along the base line KL on the right of the spot G of the mixture, leaving a gap of at least one inch from this point and from the free edge DJ. The paper sheet was then folded at I′ and II′ and was suspended in the same way as before from the trough containing the second solvent in another chamber. After allowing this solvent to run down the paper to the edge CD, it was air dried. The sector ABIJ was then attached to the sheet ICDJ in its original position by means of adhesive tape. The entire chromatogram was then sprayed with 0.25 % ninhydrin solution in acetone and heated at 60° for 10 min. The amino acids were revealed on the paper as coloured spots.

The relative mobility of the reference amino acids on the chromatogram with each solvent system was measured in terms of R_L, which represents the ratio between the distance travelled by an amino acid and that travelled by leucine. Measurement of R_L was more convenient than that of R_F since the solvent was often allowed to run beyond the free edge of the paper and to drip down in order to bring about wide separation of the amino acids. The R_L value of an amino acid for each of the solvent systems was found to vary from one chromatogram to another (Table I) but not on the same chromatogram. In view of this, the net position of the amino acids of the mixture on the sector KCHG could be readily compared with the position of the references moved by the first solvent over the sector EBIF and also with those moved by the second solvent over the sector GHDL. On this basis, the identity of the amino acids in the mixture could be established with greater certainty.

The R_L values of different amino acids for the two solvent systems were found to differ in such a manner (Table I) that spots of almost all the amino acids of the mixture subjected to two-dimensional chromatography, were quite separate and could be readily distinguished. Certain amino acids, however, had R_L values that were very close together; for instance, lysine and asparagine were close to each other. This was

also true for glycine and proline. Nevertheless, even these amino acids could be separated sufficiently by allowing the solvent to run long enough for leucine to travel about 10.5 inches from the base line in each direction.

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Simplified analysis of light gas mixtures with gas chromatography

The analysis of light gas mixtures including hydrogen, oxygen, nitrogen, methane, carbon monoxide and carbon dioxide is a common problem in gas chromatography. The difficulty in this simple analysis is that only a molecular sieve column can separate oxygen and nitrogen, but at the same time, this column, under normal operating conditions, irreversibly adsorbs carbon dioxide; on the other hand, a silica gel column which is adequate for the other part of the given mixture, does not separate oxygen and nitrogen.

In practice, multiple analysis or column combinations are used to solve this problem. In multiple analysis, two separate runs are made, one on a silica gel and the other on a molecular sieve column; in such cases, either two instruments must be used or the respective column must be changed after the first run.

To overcome the difficulty of the series operation, a special solution was suggested[1] three years ago, where one thermistor bead was installed between the two columns (the silica gel being the first) and another bead after the molecular sieve column. Thus, the carbon dioxide peak is also recorded before it is adsorbed on the second column. Of course, the disadvantage of this system is that carbon dioxide contaminates the molecular sieve, reducing its life. Similar systems were also described in more recent publications[2].

Another possibility for column combination is the use of the two columns in parallel[3]. However, even in this case, the molecular sieve will become slowly contaminated by CO_2. Another disadvantage of this method is that a proper and constant flow splitting is essential to the success of the method.