Over small pressure ranges there exists a linear relationship between pressure applied and output voltage. As the applied pressure increases, the calibration curve becomes hyperbolic in form since the plates no longer approximate a parallel relationship due to increased angular displacement of the spiral. This fact cannot be counted as too great a disadvantage, since once an accurate calibration curve has been obtained it remains valid for a considerable period of time.

The zero drift of the instrument is less than 1% over a period of 24 hours and its stability to ambient temperature changes is sufficient not to require any thermostatic control.

Over a pressure range from 0 to 10 cm of mercury the average voltage output from the transducer is 0.2 v per cm of mercury pressure. When this voltage is applied through an attenuator to a 10-mv, full-scale recorder it is evident that very high sensitivities can be obtained. The usable sensitivity is limited by the noise produced by vibration of the spiral. The maximum noise level is equivalent to 0.05 cm of mercury, and by suitable location and mounting of equipment could be reduced to less than that equivalent to 0.01 cm. The experiments in which we used the spiral manometer did not require pressure measurements of precision greater than 0.05 cm and hence no special mounting of the gauge was necessitated although, on occasion, when heavy motors were operating in the vicinity of the gauge, the noise brought about by building vibration was troublesome.

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APPLICATION OF THE VARRENTRAPP REACTION TO THE PREPARATION OF LONG-CHAIN DICARBOXYLIC ACIDS

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Aliphatic dicarboxylic acids with 11 or more carbon atoms were required for gas–liquid chromatographic identification of similar materials produced by ozonolysis of marine-oil fatty acids. Aside from brassylic acid, prepared by oxidative fission of erucic acid, these acids are not conveniently available. As an alternative to individual syntheses, modification of the Varrentrapp reaction (1–4) by limiting it to redistribution of the 13:14 double bond in erucic acid into adjacent positions, followed by oxidative fission and recovery of the dicarboxylic acids, offered a means of obtaining a number of these acids in one preparation.

The product of alkali fusion at 320° C was selected for oxidative fission as the iodine value of 60 indicated that the Varrentrapp reaction proper, production of the saturated acid two carbon atoms shorter than the original monounsaturated acid, had proceeded only to a limited extent. Since double bonds migrating to the 6:7 or 7:8 positions rapidly participate in the final stages of this reaction (2), it was expected that the residual double bonds in the fusion product would be in positions more remote from the carboxyl group.

The petroleum-ether-soluble ozonolysis product contained chiefly monocarboxylic acids such as palmitic, stearic, and arachidic, with appreciable amounts of odd-numbered monocarboxylic acids of 11 or fewer carbon atoms. The methanol-soluble ozonolysis product contained approximately 60% of roughly equal amounts of the dicarboxylic acids with 7 to 16 and 17 carbon atoms and 40% of monocarboxylic acids with 7 to 16 carbon atoms (Fig. 1).

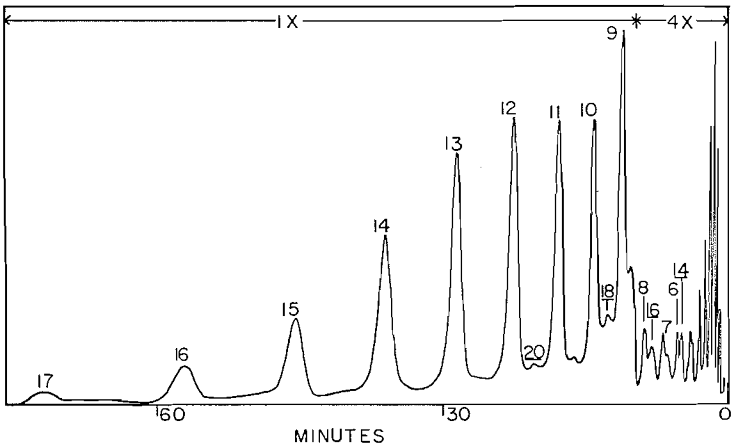

FIG. 1. Gas–liquid chromatogram of methyl esters of dicarboxylic acids. Column, 10 ft$\times\frac{1}{4}$ in., packed with 25% LAC-3R-728 on 60–80 mesh Chromosorb. Analysis of 0.005 ml at 200° C with hydrogen carrier gas flowing at 80 ml/minute. Numerals indicate the number of carbon atoms in the acid chains, monocarboxylic acids being underlined.

Since double bonds are increasingly less susceptible to the Varrentrapp reaction when more remote from the carboxyl group, a higher proportion of long-chain dicarboxylic acids with 15 or more carbon atoms could be obtained by prolonged fusion at lower temperatures. The over-all dicarboxylic acid yield would, however, be diminished as a consequence of further completion of the Varrentrapp reaction.

EXPERIMENTAL

A technical erucic acid, iodine value 85, was employed in this reaction. Gas–liquid chromatography indicated a composition of 72.8% erucic acid, 12.4% eicosenoic acid, 6.7% oleic acid, and minor amounts of palmitic, stearic, linoleic, and arachidic acids. Alkali fusions were carried out in a covered iron vessel fitted with a nitrogen inlet and a stirrer of iron pipe. Equal weights of erucic acid, sodium hydroxide, and potassium hydroxide were placed in this vessel, and it was then placed in a Wood's metal bath preheated 40° C above the reaction temperature. The mixture was stirred continuously for $\frac{1}{2}$ hour and intermittently for a further half hour. The cooled reaction mass was dissolved in water, acidified, and the fatty acids extracted with petroleum ether. Weight recoveries were approximately 80%, with iodine values of 38, 60, and 69 from fusions at, respectively, 360, 320, and 300° C for 1 hour. Gas–liquid chromatography indicated almost complete destruction of the oleic, linoleic, and eicosenoic acids, the products thus consisting chiefly of saturated acids and docosenoic acids.

The alkali fusion product of iodine value 60 (5 g) was ozonized in glacial acetic acid and the ozonide decomposed with hydrogen peroxide (2). The final solution was diluted to 400 ml with water and extracted with diethyl ether (400 ml). The ether solution was washed with water (50 ml), and the aqueous phases discarded. The ether was removed and the product (5.7 g) was dissolved in 90% methanol (200 ml) and extracted with petroleum ether (b. p. 40–60° C, 200 ml). The petroleum-ether-soluble material (0.9 g) and the methanol-soluble material (4.8 g) were converted to methyl esters with diazomethane. Identification of certain components was effected through mixed gas–liquid chromatograms with authentic materials employing both silicone grease and polyester substrates. The identification of the remainder was confirmed by plots of logarithm retention time vs. number of carbon atoms.

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AMINO ACID DISTRIBUTION IN BIOLOGICALLY ACTIVE PEPTIDES

II. DIPEPTIDES HAVING LOW STATISTICAL SIGNIFICANCE

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In the previous publication of this series (1), the amino acid sequences of a number of biologically active proteins were examined for the occurrence of relative chain position preferences of the individual constituent amino acids and for statistically significant repeating amino acid sequences comprising dipeptidic couplet units. Thirty-five dipeptide structures were found for which the chi-square values exceeded the 90% confidence limits for one degree of freedom (2.706). The study is now extended to dipeptide sequences which occur at a very low level of statistical significance.

A total of 10 couplets, listed in Table I, occurred only once in the proteins included in

TABLE I

Dipeptide couplets having low chi-square values

Dipeptide couplet	χ^2 *	Dipeptide couplet	χ^2 *
Ala–Phe	0.000	Cys–Lys	0.013
Asp–Gly	0.003	Cys–Pro	0.013
Asp–Lys	0.000	Gly–Phe	0.015
Cys–Ser	0.001	Leu–Cys	0.013
Glu–Gly	0.002	Lys–Cys	0.013
Glu–Thr	0.000	Lys–Thr	0.016
Leu–Asp	0.000	Phe–Asp	0.023
Pro–Asp	0.000	Phe–Tyr	0.023
Pro–Tyr	0.000	Ser–Asp	0.019
Tyr–Val	0.003	Ser–Gly	0.013
		Ser–Val	0.015
Asp–Ser	0.023	Thr–Lys	0.019
Asp–Phe	0.023	Tyr–Phe	0.023
Cys–Leu	0.013	Val–Ala	0.015

*The chi-square value is calculated from the formula, $\chi^2 = \Sigma\,(A - C)^2/C$, where A is the "actual" occurrence of a particular dipeptide unit and C is the "chance" occurrence of that couplet.

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