At a $5 \times 10^{-5}M$ concentration of resorufin acetate in tris buffer, 0.01M, pH 7.4, an increase in the concentrations of cholinesterase added, from 0.00185 to 0.188 units per 3.0 ml. of total solution (or 0.00062 to 0.62 units/ml.), produces a linear increase in the rate of production of resorufin, $\Delta F/\Delta t$, allowing determination of the enzyme with a standard deviation of about ± 1.0. Even at the extreme low concentration of cholinesterase, 0.00062 units/ml., the rate of spontaneous hydrolysis was only about 10% of the enzymic rate. Previously reported minimal measurable horse serum cholinesterase concentrations were of the order of 0.01 units/ml. (*1*, *2*, *4*). The present substrate permits the assay of concentrations as low as 0.0006 units/ml. Variation in the concentrations of phosphatase, α-,β-, and γ-chymotrypsin also produced a linear change in the rate of reaction, as expressed as $\Delta F/\Delta t$, allowing measurement of these enzymes. Since resorufin concentrations as low as $10^{-8}M$ give a measurable fluorescence reading, approximately 100–1000 times less product need be formed from the hydrolysis of a fluorogenic substrate than from a chromogenic one. Hence, much lower enzyme concentrations can be assayed, fast enzymic reactions can be followed by this technique (since only low enzyme concentrations need be used), and true initial rates of reaction may be measured, since only a very small change in substrate concentration ($10^{-8}M$), gives a significant change in fluorescence. This method also provides a means of measuring lower inhibitor concentrations, since one is able to measure low enzyme and substrate concentrations.

Complete details of this procedure, together with a further discussion on the effect of the acyl chain length on the rate of enzymic hydrolysis, will be published in the near future.

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Table II. Hydrolysis of Resorufin Acetate by Horse Serum Cholinesterase

Resorufin acetate, M	Buffer	pH	$\Delta F/\Delta t$ units/minute
5×10^{-5}	Tris, 0.01M	7.4	1.20
1×10^{-5}	Tris, 0.01M	7.4	0.34
1×10^{-6}	Tris, 0.01M	7.4	0.039
1×10^{-6}	Tris, 0.1M	8.0	0.090
1×10^{-7}	Tris, 0.1M	8.0	0.012
1×10^{-8}	Tris, 0.1M	8.0	0.006

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New Derivatives for Characterization of Organic Acids

SIR: Carboxylic and sulfonic acids generally lend themselves less readily than other compounds to preparation of derivatives (the most common ones being amides or esters) for qualitative organic analysis. Many such derivatives require preparation of an acid chloride intermediate and must be crystallized one or more times to be suitable for characterization. A list of references cited in a treatise on the subject indicates great interest in the characterization of organic acids (*1*).

Our investigations, simultaneously in the indole field and in selected problems of natural compound isolation and characterization, have provided the opportunity to come upon a new, useful organic acid derivative. When stoichiometric amounts of 5-methoxytryptamine and 5-methoxyindoleacetic acid were mixed in ether and chloroform, a quantitative yield of a sharp melting crystalline salt was obtained which had a melting point of narrower range than either reactant.

Therefore, 5-methoxytryptamine was similarly reacted with a variety of other carboxylic acids and a sulfonic acid. In each instance, a useful, crystalline, sharp-melting salt derivative was obtained. Most of these salts, without crystallization, were of high

Table I. Salts of 5-Methoxytryptamine[a] and Various Acids

Acid	M.p.[b], ° C., acid	M.p., ° C., salt	Formula	Anal. data: Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
Oxalic	192 (dec.)	170–172	$C_{13}H_{18}N_2O_5$	55.3	6.4	10.0	55.8	5.9	10.0
Mandelic	119–121	156–159	$C_{19}H_{22}N_2O_4$	66.7	6.4	8.1	65.6	6.6	8.2
p-Methoxyphenylacetic	70–72	169–170	$C_{20}H_{24}N_2O_4$	67.2	6.7	7.8	66.9	7.1	7.9
Acetic	Liquid	141–143	$C_{13}H_{18}N_2O_3$	62.0	7.2	11.1	61.9	7.3	11.2
Cyanoacetic	...	166.5–168	$C_{14}H_{17}N_3O_3$	61.0	6.2	15.2	61.1	6.2	15.3
p-Toluenesulfonic	104–106	183–187	$C_{18}H_{22}N_2O_4S$	59.6	6.1	7.7	59.6	6.2	7.8
Phenoxyacetic	98–99	173–176	$C_{19}H_{22}N_2O_4$	66.7	6.4	8.1	66.4	6.3	8.3
trans-Cinnamic	132–134	178–180	$C_{20}H_{22}N_2O_3$	71.0	6.5	8.2	71 5	6.8	8.5
3-Nitrosalicylic	146–148	202–203	$C_{18}H_{19}N_3O_6$	57.9	5.0	11.2	57.5	5.2	11.1
Octanoic	Liquid	114–115	$C_{19}H_{30}N_2O_3$	68.2	8.9	8.4	68.0	9.3	8.3
Stearic	68–69.5	94–97	$C_{29}H_{50}N_2O_3$	72.8	10.5	5.9	72.9	10.4	6.5
Benzoic	123–125	160–161	$C_{18}H_{20}N_2O_3$	69.2	6.4	8.8	69.1	6.5	8.9
5-Methoxy-3-indoleacetic	147–150	181.5–183	$C_{22}H_{25}N_3O_4$	66.8	6.3	10.6	66.5	6.5	10.8
3-Indoleacetic	168–170	192–193	$C_{21}H_{23}N_3O_3$	69.0	6.3	11.5	68.5	6.4	11.4

[a] Melting point, 120°–123° C.
[b] Carried out in *Mel-Temp* apparatus corrected against standards.

purity as indicated by their analytical values (Table I). Although in most cases crystallization was not necessary for purification, several of the salts could be crystallized in good yields from ethyl acetate. The salts were not hygroscopic or otherwise sensitive to the atmosphere, so special precautions need not be taken with solvents or in isolation and drying.

One illustration of the usefulness of this type of derivative was in connection with recovery of indoleacetic acids from a thin layer chromatogram. When 5-methoxyindoleacetic acid was chromatographed on a thin layer of silica on a glass plate, then recovered from the silica, it lost its characteristic infrared spectrum, indicating extensive degradation during chromatography although a discrete spot was obtained. However, a 5-methoxytryptamine salt prepared from an identical fraction gave an infrared spectrum matching that of the authentic salt. Both isolation and a high degree of purification were easily effected in one step.

A further advantage of the salt derivative described here was that very small amounts of acid could be converted. It was easily possible to start with 5 mg. of acid and recover a quantitative yield of derivative without using any special equipment. Of 15 acids tried, only salicylic acid did not form a precipitate even after standing several days, though 3-nitrosalicylic produced a salt immediately in quantitative yield. The salts of the longer chained fatty acids, stearic and octanoic, were soluble in the chloroform-ether solutions used. However, when large quantities of *n*-heptane were used in the preparations, salts could also be obtained in quantitative yields as gelatinous precipitates.

EXPERIMENTAL

Preparation of Salts of 5-Methoxytryptamine and Organic Acids. A stock of 5-methoxytryptamine, m.p. 120° to 123° C. (Regis Chemical Co., Chicago 10, Ill.), in chloroform was used (10 mg. per ml.). In one instance, 3.9 ml. of the stock solution was added to 41 mg. of 5-methoxy-3-indoleacetic acid dissolved in 2 ml. of ether (used without drying). An immediate precipitate formed and the mixture was cooled for 15 minutes in ice, filtered, and the salt dried in air. All yields were quantitative.

A slight departure in procedure was used for longer chain fatty acids, octanoic and stearic. Thus, 3.9 ml. of 5-methoxytryptamine (10 mg. per ml.) in chloroform were added to 0.3 ml. of octanoic acid in 110 ml. of *n*-heptane. A gelatinous precipitate formed and the mixture was cooled at 5° C. overnight to obtain 60 mg. of the pure salt on vacuum filtration. A stearic acid salt was made in a similar manner, but it was too gelatinous to filter and was isolated by centrifugation.

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Detection of Dichlorophene, Hexachlorophene, and Other Related Bisphenols by Gas Liquid Chromatography

SIR: A gas liquid chromatographic method has been developed which permits the investigation of common halogenated bisphenols, particularly those found to have commercial application. Conditions have been devised so that these high melting and even higher boiling materials can be injected and eluted from a column in symmetrical peaks with no noticeable decomposition (Figure 1). The novelty of the technique as here presented is the use of short columns in the range of 8 to 12 inches, moderately high temperatures (200° to 250° C.), relatively short retention times (5 to 15 minutes), and fast flow rates (100 to 150 ml. per minute). This technique is of special value in mixtures since the methods in current use have shortcomings in this respect. The colorimetric methods (*5*, *8*, *9*) are not specific for any one member in the class.

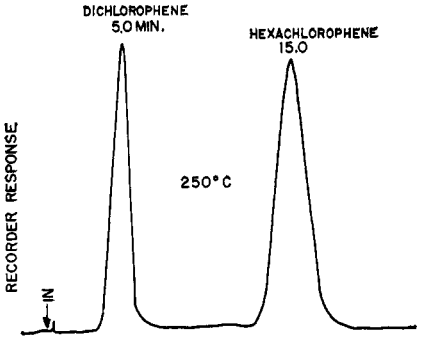

Figure 1. Typical chromatogram

Ultraviolet absorption is the other approach, but these methods (*3*, *7*, *10*) are often useless in the presence of interferences. Derry, Holden, and Newberger (*4*) have employed liquid-liquid partition chromatography with subsequent ultraviolet determination of the eluates. They have applied the method to synthetic dichlorophene–hexachlorophene mixtures and to cosmetics with some success. The separations are not sharp and the subsequent ultraviolet determinations are still subject to the limitations of that method. Bravo and Hernández (*2*) have developed paper and plate chromatographic methods for resolving mixtures of dichlorophene and hexachlorophene. In the paper method, detection is made with ferric chloride and potassium ferricyanide while ultraviolet measurement is used after elution from the plates. It is now possible with gas-liquid chromatography to detect bisphenols by direct injection of many cosmetic or pharmaceutical preparations containing them.

EXPERIMENTAL

An F&M model 609 flame ionization unit was used in this work with helium carrier gas. A 1/4-inch o.d. glass column 12 inches long was made into U-tube shape and filled with a packing made of 10% DC-710 silicone oil on Chromport

Table I. Relative Retention Data

Common name	Chemical name	Relative retention
...	2,2′-Methylenedi-*p*-cresol	0.38
...	2,2′-Thiobis(4-chlorophenol)	0.61
Dichlorophene (G-4®)	2,2′-Methylenebis(4-chlorophenol)	1.00
Bithionol	2,2′-Thiobis(4,6-dichlorophenol)	1.30
Hexachlorophene (G-11®)	2,2′-Methylenebis(3,4,6-trichlorophenol)	3.00