from linearity, which may be attributed to the gradual decrease in the diffusion during the later part of the exchange reaction. From the initial slope of the plot, the value of k was calculated.

RESULTS AND DISCUSSION

The values of the rate constant, k, were calculated using the procedure given above over a range of values of b from about 1.1 to 0.12 meq./l. It was observed that the value of k obtained was practically independent of the value of b. The value of k was also within about $\pm 5\%$ for the four cinchona alkaloid sulfates studied for each resin used. The values of k thus obtained are given for resins $X1$, $X2$, and the three particle sizes of $X4$ in Table I at 35 and 45°. The value of the apparent energy of activation for each of these resins was evaluated according to the Arrhenius equation (7) and is almost the same, 3.06 kcals.

Similarly, runs were carried out at 35 and 45° for the three particle sizes of the resin (Amberlite-200) with four cinchona alkaloid sulfates and the same conclusions were obtained. The values of k are given in Table I. The value of apparent energy of activation is higher, and is 5.19 kcals. The value of k is inversely proportional to the average particle diameter, a, as indicated by the values of k and a.

The observation that the value of k is almost the same for the four cinchona alkaloid sulfates indicates that for the expanded structure resin, the rate of exchange is practically the same although the value of P_R (which gives the value of equilibrium exchange) (2) is higher for cinchonine sulfate and cinchonidine sulfate than that for quinine sulfate and quinidine sulfate.

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Polyamide-Kieselguhr Thin-Layer Chromatography of Antioxidants

HUNG-CHEH CHIANG and REN-GUU TSENG

Abstract □ Eight fat antioxidants are identified on the polyamide-kieselguhr G (2:1) mixed layer, which is firmly bonded and easy to handle. The sharp separation is achieved by use of both aqueous and nonaqueous solvent systems.

Keyphrases □ Antioxidants—identification □ TLC—separation, identification □ UV light—TLC spot visualization □ Iodine vapor—TLC spot visualization

Thin-layer chromatography of fat antioxidants has been the subject of numerous investigations. The separation by TLC on alumina (1), silica gel (2, 3), silica gel-kieselguhr mix layer (4), acetylcellulose (5), polyamide (6), and polyamide-starch mix layer (7), has been reported, but the application of a mixed polyamide-kieselguhr layer in the chromatographic separation has not. In this note, the separation of eight fat antioxidants by this mixed layer procedure is described.

EXPERIMENTAL

Materials—The solvents and chemicals are reagent grade (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Thin-Layer Plates—Twenty-two grams of polyamide[1] chip was dissolved in a mixture (80:20) of 90% formic acid and distilled water. After obtaining a homogeneous solution with gentle warming and stirring, 10 g. of kieselguhr G (E. Merck) was added. Two hundred milliliters of the above solution was added to a dish (14.5 × 19.5 × 2.5 cm.) and a glass plate (12 × 16 × 0.1 cm.) dipped into it. Both sides of the glass were covered evenly. The glass was placed over the dish for 2 min. to let the excess solution drain. It was then air-dried for 3 hr. and heated at 100° for 30 min.

Chromatographic Procedure—A 0.5% alcoholic solution of antioxidants was applied to the start line 1.5 cm. from the bottom of the layer and developed by ascending techniques. The chamber was equilibrated with the respective solvent for 30 min. before use.

Visualization—The layers were sprayed with 0.07% rhodamine B alcoholic solution and deep violet spots were observed under the

[1] Nylon 6, type 1022B of UBE Industrial Ltd., Osaka, Japan. U. S. supplier: American Enka Corp.

Table I—Chromatographic Data

	Group				
	N				*A*
	Solvent[a]				
	I	II	III	IV	V
Substance	Time, hr.[b] 9	2	2.5	2.5	3
n-Propyl gallate	0.26	0.22	0.27	0.63	0.63
Isoamyl gallate	0.36	0.31	0.33	0.61	0.52
Lauryl gallate	0.56	0.54	0.48	0.44	0.45
Cetyl gallate	0.64	0.65	0.53	0.21	0.19
Stearyl gallate	0.72	0.67	0.56	0.08	0.08
Butylated Hydroxyanisole	0.69	0.87	0.74	0.70	0.54
Butylated Hydroxytoluene	0.80	0.95	0.63	0.10	0.14
Ethyl protocatecuate	0.42	0.38	0.37	0.55	0.64

[a] I, isoamyl alcohol; II, isoamyl acetate–acetone (5:1); III, isoamyl acetate–xylene–ethanol (20:1:1); IV, acetone–water (5:3); V, dioxane–water–ethanol (10:7:5). [b] Required to ascend 10 cm. from origin.

UV light. The spots were detected by exposing the plate to iodine vapor.

RESULTS AND DISCUSSION

R_f values obtained with the five solvent systems are given in Table I. It is very interesting to note that the R_f values of the gallic acid ester in the two solvent groups (*N* and *A*) are reversed.

In a nonaqueous solvent system (Group *N*), the R_f values of the gallic acid ester increase with an increase in length of the carbon chain. In this case, the separation is mainly based on the adsorption or partition between the kieselguhr and the ester. In aqueous solvent systems (Group *A*), the R_f values decrease with an increase in length of the carbon chain. The separation mechanism may depend on the reversible formation of hydrogen bonds between the carbonyl-oxygen atom of polyamide and the hydrogen atom of phenolic group in the ester.

The layer is firmly bonded, does not crack, and can be stored easily. Both sides of the glass are independent of each other and chromatography can be performed simultaneously on each side.

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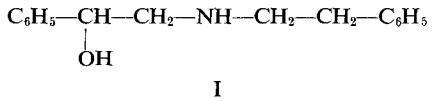
Fate of 2-Phenethylamino-l-phenylethanol,2-^{14}C in Rats

J. N. WELLS and W. G. ANDRUS, Jr.*

Abstract □ The fate of 2-phenethylamino-1-phenylethanol,2-^{14}C was determined following i.p. administration to rats. Little if any radioactivity was detected in tissue samples 2 days after dosage. The radioactivity was eliminated primarily in the urine. Recovery amounted to 91% in the urine and 6.8% in the feces after 3 days. The radioactivity in the urine was due to the administered compound. LD_{50} determination and *in vivo* MAO inhibition are also reported.

Keyphrases □ 2-Phenethylamino-1-phenylethanol—toxicity, *in vivo* inhibition and metabolic fate □ LD_{50} determination—2-phenethylamino-1-phenylethanol

2-Phenethylamino-1-phenylethanol (I) has been reported (1) to be a good inhibitor of monoamine oxidase (MAO) *in vitro*. The purpose of this study was to determine the toxicity, *in vivo* MAO inhibition, and metabolic fate of I.



I

EXPERIMENTAL

Methods and Materials—The LD_{50} of I was determined by the method of Horn (2). *In vivo* MAO inhibition was studied using the reserpine reversal technique (3). A liquid scintillation counter[1] was used to measure radioactivity of all samples. The background count rate of the instrument was stable at 25 to 30 c.p.m. throughout the study. Two counting formulations were employed: XDC scintillator for counting water-soluble samples consisted of 1 part xylene, 3 parts 2-ethoxyethanol, 1.0% 2,5-diphenyloxazole, 0.05% 1,4-bis-(4-methyl-5-phenyloxazolyl) benzene, and 8.0% naphthalene; TC scintillator for counting feces and tissue samples consisted of 1 part toluene, 1 part 2-ethoxyethanol, and 0.6% 2,5-diphenyloxazole. All counted samples were fortified with ^{14}C-toluene internal standard (15λ of 0.77 μc./ml.) and recounted to determine counting efficiency. All samples were counted in low-potassium counting vials.[2]

Thin-layer chromatograms (250 μ) spread with Silica Gel G[3] were activated at 110° for 0.5 hr. prior to use. Solvent systems used were ethanol–ammonium hydroxide (4:1) ethanol–acetic acid (4:1) and chloroform–methanol–water (75:22:3).

Labeled compounds were identified from autoradiograms using medical X-ray film.[4] Exposure times were based on exposure to the film of 10^8 disintegrations so that concentrations of 1% could be detected.

Reagent grade chemicals and ^{14}C-styrene[5] were used as received.

2-Phenethylamino-1-phenylethanol,2-^{14}C—Perbenzoic acid (30 ml. of a 15% solution in benzene) was placed in a 100-ml. three-necked flask equipped with stirrer and condenser. The flask was cooled to 0° and a solution of 1 mmole (104 mg.; 1 mc.) of 8-^{14}C styrene and 8.6 mmole (895.9 mg.) of styrene in 1.0 ml. of ether was added. After 24 hr. at 0° the solution was extracted with 3 × 30 ml. of 10% NaOH. The organic phase was washed with 3 × 30 ml. of water and then dried. Removal of the benzene *in vacuo* gave 0.85 ml. of 8-^{14}C-styrene oxide.

Table I—LD_{50} Determination of I·HCl in Mice[a]

Group[b]	Dose, i.p., mg./kg.	Deaths
1	46.4	5
2	31.6	2
3	21.5	0
4	10.0	0

[a] Male albino mice, 18–22 g. (Harland Industries). [b] Each group contained five mice.

[1] Beckman LS-100, Beckman Instruments Inc., Fullerton, Calif.

[2] Packard Instrument Co., Inc., Downers Grove, Ill.

[3] Brinkman Instruments Inc., Westbury, N. Y.

[4] No Screen, Eastman Kodak Co., Rochester N. Y.

[5] International Chemical and Nuclear Corp., City of Industry, Calif.