AN ASSAY FOR PRIMARY ARYLALKYLAMINES

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BAKER, Harbourne and Ollis[1] showed that salicyloylacetone reacted with primary aliphatic amines, but not amino-acids, to give yellow fluorescent *o*-β-alkylaminocrotonylphenols. Attempts were made to apply this reagent for estimation of 1-(*p*-alkyloxyphenyl)ethylamines without success. Salicylaldehyde was a satisfactory alternative. Formation of aldimines to discriminate between primary and alkylated amines has attracted little attention for assay purposes. Loeper[2] made reference, without details, to the use of salicylaldehyde for estimation of tryptamine, though later *m*-nitrobenzaldehyde was specified as the reagent[3].

EXPERIMENTAL

Salicyloylacetone was made by the method of Badcock, Dean, Robertson and Whalley[4]. Salicylaldehyde was purified through its bisulphite compound and twice distilled, b.pt. 40°/0·5 mm. Derivatives of these carbonyl compounds with primary amines were prepared by setting aside equimolecular proportions of the reactants in benzene. After 12 hours the solvent was removed under reduced pressure and the residue crystallised from light petroleum. New derivatives are listed in Table I.

TABLE I

DERIVATIVES OF PRIMARY AMINES WITH SALICYLOYLACETONE AND SALICYLALDEHYDE

Amine	Salicyloylacetone derivative		Salicylaldimine	
	m.pt.°C.	Analysis	m.pt.°C.	Analysis
Mescaline	119	Found: N, 3·7·$C_{21}H_{33}O_6N$ requires N, 3·8 per cent.	112	Found: N, 4·4· $C_{18}H_{21}O_4N$ requires N, 4·4 per cent.
1-(*p-cyclo*Hexyloxyphenyl)-ethylamine	95	Found: N, 3·7· $C_{24}H_{39}O_3N$ requires N, 3·7 per cent.	108	Found: N, 4·5· $C_{21}H_{33}O_2N$ requires N, 4·3 per cent.
1-(*p-iso*Propyloxyphenyl)-ethylamine	—	—	73	Found: N, 5·0· $C_{13}H_{21}O_2N$ requires N, 4·9 per cent.

The yellow fluorescence of several *o*-β-alkylaminocrotonylphenols in ultra-violet light was quenched by common laboratory solvents. Solutions in methanolic sulphuric acid developed a blue fluorescence, probably due to 2-methylchromone arising from hydrolysis and cyclisation of the salicyloylacetone produced[5]. The intensity of fluorescence corresponded to equivalent amounts of salicyloylacetone under similar conditions. It was dependent on the final concentration of acid, showing a sharp maximum at 47 per cent. v/v. Sensitivity was adequate, the fluorescence developed by pure ketimines being detectable at 0·01 μg./ml. but the yields of derivatives at high dilution were too variable and low for practical use.

Alkaline solutions of salicylaldehyde had a bright yellow colour and a

greenish yellow fluorescence in ultra-violet light. The salicylaldimines of Table I and that of amphetamine had the same characteristics, the intensity of colour corresponding to the equivalent salicylaldehyde content (Table II). There was a linear relation between concentration of aldimine, and colour or fluorescence intensity, up to 100 μg./ml. and 1 μg./ml. respectively.

TABLE II

EQUIVALENCE OF COLOUR INTENSITY DEVELOPED BY SALICYLALDEHYDE AND SALICYLALDIMINES IN ALKALI

239 parts amphetamine salicylaldimine ≡ 122 parts salicylaldehyde. Other amines mentioned in the text gave a similar result

Amphetamine aldimine		Salicylaldehyde	
Concentration (μg./ml.)	Reading	Concentration (μg./ml.)	Reading
23·9	0·279	12·2	0·285
47·8	0·554	24·4	0·561
81·7	0·810	36·6	0·817
95·6	1·05	48·8	1·08
119·5	1·29	61·0	1·29

Method of Assay

The solution of amine was acidified with a few drops of hydrochloric acid, warmed to 40° C. and carbon dioxide swept out by a current of nitrogen. The solution was adjusted to pH 9 and the base extracted by shaking five times with 5 ml. portions of volatile solvent, preferably benzene. The extract was lightly centrifuged to separate alkaline globules and the solution transferred to a flask containing about 10 molecular proportions of salicylaldehyde in benzene. By concentration to small bulk in a current of air on a sand bath the solution was dried (more benzene being added if necessary) and the aldimine formed. The volume was adjusted to 20 ml. and 5 ml. portions transferred to ground joint tubes containing 0·5 ml. 1 per cent. acetic acid in benzene. Solvent was removed completely in a current of air on a sand bath and the excess aldehyde distilled under reduced pressure at 0·5 mm. for 30 minutes with the tubes completely immersed in water at 70° C. A blank and a suitable amount of pure alkimine as standard were submitted to the same process as the extracted amine. The residues were dissolved in 5 ml. of warm absolute ethanol and 1 ml. of N sodium hydroxide added followed by 4 ml. of water. The yellow colour, developed after a brief lag, was measured at 415 mμ.

Modifications. The procedure required slight modification according to the amine being assayed. Mescaline was preferably extracted continuously with benzene for 12 hours. Amphetamine salicylaldimine, a yellow liquid b.pt. 140–150° C./0·4 mm. (bath temp.) was appreciably volatile at 70° C./0·5 mm. It was assayed satisfactorily by evacuating at 40° C./0·5 mm. The principle source of error was contamination of the initial solvent extract with alkali. This trapped salicylaldehyde and prevented its distillation. For *p-cyclo*hexyloxy-1-phenylethylamine this contamination could be removed by washing the ether extract with water,

but even a single wash would remove up to 30 per cent. of mescaline or the *iso*propyl ether (Table III). Centrifugation and the acetic acid additive were found to be adequate safeguards.

Sensitivity. Colour measurement was applicable to solutions containing not less than 10 μg./ml. salicylaldehyde equivalent. Below this concentration fluorescence measurement was applicable, with a practical lower limit of 0·1 μg./ml. The fluorimetric method was not developed fully due to difficulty in stabilising the mains supply to the ultra-violet source.

TABLE III

RECOVERY OF AMINES FROM 0·10 PER CENT. SOLUTIONS OF THEIR SALTS

Solutions of the salts at pH 9 were extracted three times with solvent and estimated colorimetrically as described in the text

Amine	No. of expts.	Solvent	Solution	Total mg.	Recovered mg.
1-(*p-cyclo*Hexyloxyphenyl)ethylamine	4	ether	water	10	9·9–10·0
	4	ether	water	1	0·85–0·92
	4	ether	human urine	1	0·87–0·89
	2	ether	rat urine	1	0·80–0·82
1-(*p-iso*Propyloxyphenyl)ethylamine	9	ether	water	10	4·82±0·92*
	3	ether	water	10	8·9–9·4
	2	ether	human urine	10	8·8; 9·2
Mescaline	4	chloroform	water	10	7·7–8·4*
	4	chloroform	water	10	9·9–10·3
	2	benzene†	water	10	10·0; 10·0
Amphetamine	3	benzene	water	10	7·9–8·2
	1	benzene	human urine‡	49	52

* *Low recovery due to washing the solvent extract.*
† Continuous extraction overnight.
‡ Supplied by Dr. Connell who found 49 mg. by the method of Brodie and Udenfriend[4].

RESULTS

Table III gives the recovery of amines added to water and to urines using the colorimetric process. Ammonia, acetone, acetoacetic ester and glycine did not interfere but bicarbonate and carbon dioxide could markedly reduce the proportion of amine recovered (Table IV). Secondary

TABLE IV

RECOVERY OF 1-(*p-cyclo*HEXYLOXYPHENYL)ETHYLAMINE FROM SALINE SOLUTIONS

10 mg. of the hydrochloride in 10 ml. saline solution with or without passage of 5 per cent. carbon dioxide, basified with 5-N sodium hydroxide. Ether extract estimated. Similar results were found with 1-(*p-iso*propyloxyphenyl)ethylamine and mescaline

Salt	Passage of carbon dioxide	Recovery per cent.
Sodium bicarbonate, 0·5 g.	+	27±3·4
Sodium bicarbonate, 0·5 g.	−	48; 50
Potassium bicarbonate, 0·5 g. ..	−	48
Potassium bicarbonate, 0·5 g. ..	+	28*
Sodium carbonate (hydrated) 1·0 g.	−	94
Sodium dihydrogen phosphate, 0·5 g.	+	94

* The aqueous residue acidified and freed of carbon dioxide, then basified and re-extracted raised recovery to 100 per cent.

amines e.g., methedrine, and tertiary amines, e.g., methadone, gave deep orange colours with salicylaldehyde but these disappeared during the vacuum distillation and low readings (0 to 3 per cent. apparent recovery) were obtained. Amines of low molecular weight, e.g., *iso*amylamine gave aldimines that were volatile at 70°/0·5 mm. and they were lost. Aromatic amines, e.g., *p*-aminosalicylic acid, gave yellow colours on the final addition of alkali but these faded within a few minutes.

Human urine (50 ml.) gave small positive readings. Of sixteen specimens from normal or mentally ill subjects, eight gave zero values, six gave values equivalent to the excretion of 115–264 μg. salicylaldehyde equivalent/24 hours. Two specimens from patients gave relatively high values equivalent to 1·23 and 1·53 mg. salicylaldehyde equivalent/24 hours. Adult female albino rat urine gave ether extracts corresponding to the excretion of 145 $\pm$ 35 μg. salicylaldehyde equivalent/24 hours. Similar animals rapidly metabolised *p-cyclo*hexyloxy- and *p-iso*propyloxy-1-phenylethylamine. After giving the 1–^{14}C labelled amines there was good recovery of ^{14}C, the major fraction being excreted in 24 hours, but only low recovery of primary amine was obtained (Table V). ^{14}C–metabolites not assayable as primary amine, were difficult to extract from urine even after acid hydrolysis, whether the solution was finally made acid, alkaline or neutral. Continuous extraction for three days with ether at pH 9 took out only half the labelled material present. No anticipated metabolite was traced.

TABLE V

RECOVERY OF AMINES FROM URINE OF RATS

The dose specified was divided between two rats and given i.p. Two similar rats receive saline. Radioactivity estimated on the evaporated urine at infinite thickness. Standards were prepared by addition of amines to rat normal urine

Amine	Dose (mg.)	Collection period (days)	Per cent. recovery of primary amine		Per cent. recovery of ^{14}C
			by assay	by ^{14}C	
1-(*p-cyclo*Hexyloxyphenyl)ethylamine	10	4	7·8; 10	—	—
	10	1	2·9	4·3	55
	20	2	19	16	69
	20	1	15*	—	—
1-(*p-iso*Propyloxyphenyl)ethylamine ..	10	1	14	5·8	81
Mescaline	20	1	12	—	—
	20	2	16	—	—

* Acid hydrolysis increased this to 28·5 per cent. but a benzene soluble pink substance interfered.

SUMMARY

1. A colorimetric method for the estimation of primary arylalkylamines is described.

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REFERENCES

1. Baker, Harbourne and Ollis, *J. chem. Soc.*, 1952, 3215.
2. Loeper, *Pr. Méd.*, 1937, **45**, 1603.
3. Lesure, *J. Pharm. Chim. Paris*, 940, **1**, 55.
4. Badcock, Dean, Robertson and Whalley, *J. chem. Soc.*, 1950, 903.
5. Virkar and Shah, *Proc. Indian Acad. Sci.*, 1949, **30A**, 57.
6. Brodie and Udenfriend, *J. biol. Chem.*, 1945, **158**, 705.

Addendum to the footnote to the paper:

ORTHO SUBSTITUTED BENZOIC ACID ESTER OF DIALKYL AMINOALKANOL IN EXPERIMENTAL CARDIAC ARRHYTHMIAS

BY R. B. ARORA, V. N. SHARMA AND B. R. MADAN

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The full chemical name for the compound McN-A-29-11 is β-Diethylaminoethyl 2:3:5:6-tetramethyl benzoate.