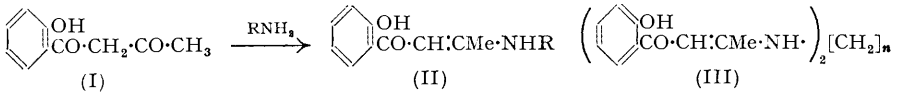
615. *Characterisation of Primary Aliphatic Amines by Reaction with o-Acetoacetylphenol and by Paper Chromatography.*

By Wilson Baker, J. B. Harborne, and W. D. Ollis.

Primary aliphatic amines may be characterised by reaction with *o*-acetoacetylphenol (I) to give crystalline *o*-β-alkylaminocrotonylphenols (II) which show an intense characteristic greenish-yellow fluorescence in ultra-violet light. Amines may be satisfactorily separated by paper chromatography, and the primary amines located as fluorescent spots after spraying with a solution of *o*-acetoacetylphenol.

It was observed by Baker and Butt (*J.*, 1949, 2142) that *o*-acetoacetylphenol (I) reacted with benzylamine and with methylamine to give yellow products which showed vivid greenish-yellow fluorescence in ultra-violet light. These products were regarded as *o*-β-alkylaminocrotonylphenols (II), and this structure has since been proved by Baker, Harborne, and Ollis (*J.*, 1952, 1294). The enamine structure (II) is preferred to that of the tautomeric ketimine because the extended conjugated system in the former is more consistent with the colour and fluorescence of these substances. This reaction is now shown to be general for primary aliphatic amines (excluding zwitterions) and may be used for their characterisation.



(I) (II) (III)

Many primary aliphatic amines, including some of biological importance such as tyramine and tryptamine (see Experimental), react with ethanolic *o*-acetoacetylphenol (I) at room temperature. Aliphatic primary diamines also react to give the expected bis-condensation products (III). All these derivatives are stable, easily purified by recrystallisation, and yield copper complexes with cupric acetate (Baker, Harborne, and Ollis, *loc. cit.*). They are well suited for the characterisation of primary aliphatic amines. *o*-Acetoacetylphenol does not react with secondary amines, primary aromatic amines, or amino-acids.

2-Acetoacetyl-5-methoxyphenol and 2-acetoacetyl-4-methylphenol (Baker and Butt, *loc. cit.*) react similarly with primary aliphatic amines to give fluorescent products, and it is clear that this is a general reaction of ω-acetyl-*o*-hydroxyacetophenones. The very ready formation of the brilliantly fluorescent *o*-β-alkylaminocrotonylphenols (II) has led to a method for locating primary aliphatic amines in paper chromatography. Amines have been separated by using *n*-butanol–acetic acid–water as the mobile phase, and after development the paper was dried, sprayed with a 1% solution of *o*-acetoacetylphenol in *n*-butanol, and the amines located by the fluorescent spots observed in ultra-violet light. This method gave good results with biologically important amines such as tyramine and tryptamine, and with various sympathomimetic amines of pharmacological interest including noradrenaline. Some naturally occurring amines have been identified previously by paper chromatography including, for example, histamine (Urbach, *Proc. Soc. Exp. Biol.*, 1948, **68**, 430; 1949, **70**, 146; 1949, **72**, 626; Block, *Anal. Chem.*, 1950, **22**, 1327) and noradrenaline (James, *Nature*, 1948, **161**, 851; Goldenberg, Faber, Alston, and Chargaff, *Science*, 1949, **109**, 534; Glazko and Dill, *Nature*, 1951, **168**, 32; Crawford and Outschoorn, *Brit. J. Pharmacol.*, 1951, **16**, 8). Recently, a method for the identification of the terminal amino-acids in proteins by conversion into amino-alcohols has been reported (Fromageot, Jutisz, Meyer, and Penasse, *Biochem. Biophys. Acta*, 1950, **6**, 283); it is suggested that these amino-alcohols might be characterised by reaction with *o*-acetoacetylphenol.

The R_F values of different amines on paper vary as expected according to their hydrophilic character. As the homologous series of primary aliphatic amines is ascended, so the R_F value increases, and the R_F value of diamines or of monoamines containing hydrophilic substituents is less than the R_F values of corresponding unsubstituted amines.

Amine	Product : R in (II)	Recryst. from : ‡	M. p.	Yield, %	Formula	Found, % : C	H	N	Required, % : C	H	N
Methyl-	* Methyl-	*e*	101°	24	$C_{11}H_{13}O_2N$	—	—	—	—	—	—
Ethyl-	*Ethyl-*	*a*	97·5	42	$C_{12}H_{15}O_2N$	70·5	7·6	6·8	70·3	7·3	6·8
n-Propyl-	n-*Propyl-*	*b*	77—78	63	$C_{13}H_{17}O_2N$	71·0	7·5	6·4	71·2	7·8	6·4
*iso*Propyl	iso*Propyl-*	*a*	89	63	$C_{13}H_{17}O_2N$	71·3	7·6	6·3	,,	,,	,,
n-Butyl-	n-*Butyl-*	*a*	106	74	$C_{14}H_{19}O_2N$	71·9	7·9	6·1	72·1	8·1	6·0
*iso*Butyl-	iso*Butyl-*	*a*	111—112	27	$C_{14}H_{19}O_2N$	72·2	7·8	6·0	,,	,,	,,
n-Hexyl-	* *n*-Hexyl-	*a*	95—96	67	$C_{16}H_{23}O_2N$	—	—	—	—	—	—
*cyclo*Hexyl-	cyclo*Hexyl-*	*a*	92	41	$C_{16}H_{21}O_2N$	74·1	8·1	5·5	74·1	8·1	5·4
Benzyl-	* Benzyl-	*a*	124	53	$C_{17}H_{17}O_2N$	—	—	—	—	—	—
n-Octyl-	n-*Octyl-*	*a*	95—97	75	$C_{18}H_{27}O_2N$	74·8	9·9	4·7	74·8	9·3	4·8
Ethanolamine ...	2′-*Hydroxyethyl-*	*f*	122	36	$C_{12}H_{15}O_3N$	65·4	6·7	6·2	65·2	6·8	6·3
† Tyramine	*Tyramino-*	*c*	147	54	$C_{18}H_{19}O_3N$	72·8	6·4	4·7	72·7	6·4	4·7
† Tryptamine ...	*Tryptamino-*	*a*	167	30	$C_{20}H_{20}O_2N_2$	74·7	6·2	8·8	75·0	6·3	8·7
Diamine											
Ethylene-	(III; $n = 2$)	*d*	183	55	$C_{22}H_{24}O_4N_2$	69·5	6·3	7·4	69·7	6·3	7·4
Trimethylene-	(III; $n = 3$)	*d*	171—172	13	$C_{23}H_{26}O_4N_2$	—	—	7·3	—	—	7·1
Tetramethylene-	(III; $n = 4$)	*d*	210 (decomp.)	44	$C_{24}H_{28}O_4N_2$	—	—	7·2	—	—	6·9
Pentamethylene-	(III; $n = 5$)	*d*	190—191	34	$C_{25}H_{30}O_4N_2$	70·9	6·9	6·7	71·1	7·1	6·6
Hexamethylene-	(III; $n = 6$)	*d*	183—184	64	$C_{26}H_{32}O_4N_2$	—	—	6·7	—	—	6·4
1 : 2-Diaminopropane	(cf. III)	*e*	187	40	$C_{23}H_{26}O_4N_2$	69·8	6·4	7·4	70·0	6·6	7·1

* These derivatives have been described previously, the first two by Baker and Butt (*loc. cit.*), and the last by Baker, Harborne, and Ollis (*loc. cit.*).

† For the reaction with *o*-acetoacetylphenol, the amine was liberated from its hydrochloride by treating a solution of the latter in ethanol with ethanolic sodium ethoxide. Tyramine, which is insoluble in cold ethanol, was treated with *o*-acetoacetylphenol in hot ethanol.

‡ Solvents used for recrystallisation : *a*, ethanol; *b*, methanol; *c*, aqueous ethanol; *d*, benzene; *e*, benzene–light petroleum; *f*, aqueous methanol.

Experimental

M. p.s are uncorrected. Analyses are by Drs. Weiler and Strauss, Oxford, and Mr. W. M. Eno, Bristol.

Reaction of o-Acetoacetylphenol with Primary Aliphatic Amines. Formation of o-β-Alkylaminocrotonylphenols (II).—The following general method was used. The amines (1·1 equiv.) alone or dissolved in ethanol were added to an ethanolic solution of *o*-acetoacetylphenol (1 equiv.; Wittig, *Annalen*, 1926, **446**, 169) at room temperature; the solutions immediately became yellow-green and were strongly fluorescent in ultra-violet light. After a few hours the *o*-β-alkylaminocrotonylphenols began to crystallise and after 2 days the solid was collected and crystallised from the appropriate solvent. The products were obtained as pale green or greenish-yellow prisms or needles which showed a very strong greenish-yellow fluorescence in ultra-violet light.

Reaction of 2-Acetoacetyl-5-methoxyphenol with Methyl-, Ethyl-, and Benzyl-amines.—The amines were treated with 2-acetoacetyl-5-methoxyphenol as in the previous cases and the products were recrystallised from ethanol. The yields are given in parentheses. Methylamine gave 5-*methoxy*-2-β-*methylaminocrotonylphenol* (60%), pale yellow plates, m. p. 157° (Found: C, 65·6; H, 7·0; N, 6·5. $C_{12}H_{15}O_3N$ requires C, 65·2; H, 6·8; N, 6·3%). Ethylamine gave 2-β-*ethylaminocrotonyl*-5-*methoxyphenol* (83%), pale yellowish-green plates, m. p. 112·5° (Found: C, 66·2; H, 7·2; N, 6·0. $C_{13}H_{17}O_3N$ requires C, 66·4; H, 7·2; N, 6·0%). Benzylamine gave 2-β-*benzylaminocrotonyl*-5-*methoxyphenol* (96%), pale green needles, m. p. 133° (Found: C, 72·6; H, 6·1; N, 4·7. $C_{18}H_{19}O_3N$ requires C, 72·7; H, 6·4; N, 4·7%). These compounds were vividly fluorescent in ultra-violet light.

Paper Partition Chromatography of Primary Amines.—The following amines have been separated on Whatman No. 1 filter-paper at room temperature, butanol–acetic acid–water mixture being used as the mobile phase. After running overnight the paper was dried, sprayed with a 1% solution of *o*-acetoacetylphenol in *n*-butanol, dried again, and examined in ultra-violet light. The amines were located by well-formed, brilliantly fluorescent spots. A list of the amines used is given below; the R_F values are recorded in parentheses.

The solvents used for the development of the chromatograms were either the upper layer obtained by mixing *n*-butanol (5 parts), acetic acid (1 part), and water (4 parts), or *n*-butanol (2 parts), acetic acid (1 part), and water (1 part).

The first solvent mixture being used, the following amines were separated: benzylamine (0·70); *n*-hexylamine (0·80); *n*-heptylamine (0·83); *n*-octylamine (0·85); *n*-decylamine (0·87). By using the second solvent mixture the following amines were separated. Those marked thus * (below) were put on the paper as either hydrochlorides or sulphates.

Monoamines: Tryptamine* (0·39); tyramine* (0·58); benzylamine (0·66); 2-hydroxyethylamine (0·48); 1-phenylethylamine (0·72); 2-phenylethylamine (0·72); 2-aminoheptane (0·76).

Diamines: Ethylenediamine (0·39); trimethylenediamine (0·39); hexamethylenediamine (0·48); decamethylenediamine (0·62); 2-amino-2′-hydroxydiethylamine (0·42).

Sympathomimetic amines and analogues:		R_F
* (3 : 4)$(HO)_2C_6H_3$·CH(OH)·CH_2·NH_2	Noradrenaline (L-Arterenol)	0·34
* (3 : 4)$(HO)_2C_6H_3$·CH(OH)·CHMe·NH_2	(Cobefrin)	0·42
* (3 : 4)$(HO)_2C_6H_3$·CH(OH)·CHEt·NH_2	(Butanefrine)	0·50
p-HO·C_6H_4·CH(OH)·CHMe·NH_2	—	0·53
* *p*-HO·C_6H_4·CH_2·CHMe·NH_2	(Paredrine)	0·64
* Ph·CH(OH)·CHMe·NH_2	(Propadrine)	0·68
* Ph·CH_2·CHMe·NH_2	(Dexedrine)	0·74

The authors thank Professor H. S. Heller and Dr. R. J. Fitzpatrick, of the Department of Pharmacology of this University, for their interest and for gifts of certain amines.

The University, Bristol. [*Received, May 3rd*, 1952.]