THE REACTION OF AROMATIC ALDEHYDES WITH PHARMACEUTICAL AMINO COMPOUNDS. PART I

BY C. G. BUTLER and P. H. B. INGLE

From the Department of Pharmacy, Bradford Technical College

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THE reaction of aromatic aldehydes with primary aromatic amines to form condensation products (Schiff's bases, anils) is well known:—

$$R'\cdot CHO + H_2N\cdot R'' = R'\cdot CH:N\cdot R'' + H_2O$$

The formation of such derivatives by reaction between some aromatic aldehydes and certain members of the sulphonamide group of drugs has been reported[1,2,3,4,5]. These investigations include an evaluation of medicinal value[1], the recording of certain physical properties[2], and a method of determination[4]. The initial purpose of the present work was to provide a rapid means of characterising sulphonamides by the preparation of a derivative which could be identified by some simple test.

Benzaldehyde, salicylaldehyde, cinnamic aldehyde, anisaldehyde, *o*- and *p*-nitrobenzaldehyde, *o*- and *p*-chlorobenzaldehyde have been shown, in certain cases, to yield crystalline derivatives with a number of the sulphonamide drugs and with some synthetic local anæsthetics.

The bright yellow or orange colour developed in the reaction (particularly with salicylaldehyde) has led to an investigation into the possibility of quantitative colorimetric determination of amino compounds by this method and results obtained up to the time of publication have also been included. This work is being continued.

METHODS

(*a*) *Materials.* Salicylaldehyde was freshly distilled under reduced pressure. Industrial spirit was used for the preparation of derivatives for recognition, and in the colorimetric work pure 95 per cent. ethanol. The "sulpha" drugs were of B.P. or B.P.C. quality where applicable, and in the following cases were supplied as pure materials by the manufacturers:—

"Irgafen"—*N*-3:4-dimethylbenzoylsulphanilamide.
"Irgamid"—*N*-dimethylacryloylsulphanilamide.
Sulphamethizole—2-sulphanilamido-5-methyl-1-thio-3:4-diazole.
Sulphafurazole—5-sulphanilamido-3:4-dimethyl-*iso*oxazole.

Tablets of sulphasomidine (6-sulphanilamido-2:4-dimethylpyrimidine) were used for the preparation of the salicylaldehyde derivative.

(*b*) *Benzaldehyde derivatives.* 0·25 g. to 1 g. of the sulphonamide was dissolved in 5 ml. (or a sufficient quantity) of 95 per cent. ethanol by boiling and 1 ml. of benzaldehyde added. The mixture was heated in a boiling water bath for 5 minutes, during which a yellow colour developed. After cooling, the inside of the test tube in contact with the liquid was

scraped to induce crystallisation if this had not already occurred. The test tube was allowed to stand for a further 5 minutes to complete crystallisation, the crystals were filtered off, washed with small portions of acetone until no odour of benzaldehyde remained, and dried at 100° C. Recrystallisation was carried out using ethanol or a mixture of ethanol and benzaldehyde.

Melting points were determined by the capillary tube method, using standard 50 mm. immersion thermometers of medium range, in a hand stirred bath of dibutyl phthalate, and may thus be regarded as approximating very closely to "corrected" melting points.

The crystals were examined microscopically after mounting in a mixture of equal parts of glycerol and ethanol.

In some cases it was found necessary to use a modification of the above method, on account of either the poor solubility of the sulphonamide in ethanol or the failure of the derivative to crystallise. The following were found suitable:—

(i) The use of added benzaldehyde to increase the solubility of the sulphonamide in ethanol.

(ii) Heating the sulphonamide and benzaldehyde together on a boiling water bath in the presence of a condensing agent, such as anhydrous zinc chloride. The resulting viscous yellow liquid was mixed with 5 ml. of ethanol and on scratching the side of the tube, crystals were deposited.

(*c*) *Salicylaldehyde derivatives.* About 0·2 g. of the sulphonamide was heated to boiling with a 5 per cent. solution of salicylaldehyde in 95 per cent. ethanol. The test tube was set aside to cool until a crystalline deposit formed. This appeared after times varying from one minute to several hours. The somewhat lengthy refluxing process which has been described[2] does not appear to be essential for satisfactory yields. After a further period of standing to complete precipitation the crystals were filtered off by suction, washed with successive portions of cold ethanol until the odour of salicylaldehyde was no longer apparent. The product was dried at 100° C. and its melting point determined.

(*d*) *Derivatives with cinnamic and other aldehydes.* The method described under salicylaldehyde appeared to work satisfactorily in a large number of cases. A detailed examination is being carried out.

(*e*) *Quantitative application of the salicylaldehyde reaction.* Initial experiments to determine the conditions for complete development of colour were carried out using a single cell photoelectric absorptiometer giving direct readings based on the photocell output. Parallel experiments were made to determine the time for completion of colour development and the excess of salicylaldehyde required to achieve this in a reasonable time. The visible absorption spectrum of the solution was plotted in a 1 cm. cell using the Hilger "Uvispek" spectrophotometer and this showed the expected considerable absorption in the region of 4500 Å (Fig. 1), hence the filter selected was the Ilford 601 (Spectrum Violet). A duplicate run on a fourfold diluted solution in a 4 cm. cell gave an almost identical absorption spectrum, showing that the compound follows Beer's law.

Colour development time. A solution containing 1 g. of sulphadimidine in 250 ml. of 95 per cent. ethanol was made. 10 ml. portions of this were introduced into a series of boiling tubes and to each tube was added 10 ml. of a 10 per cent. solution of salicylaldehyde in 95 per cent. ethanol. A piece of porous pot was added and the tubes were heated in a boiling water bath, the tubes being supported in such a way that only the bottom inch was immersed. At regular intervals a tube was removed, the contents rapidly cooled and transferred to a 20-ml. graduated flask. The volume was adjusted to 20 ml. and the optical density of the coloured solution compared with that of a blank solution made by mixing equal volumes of the 10 per cent. salicylaldehyde solution and 95 per cent. ethanol. (This solution on heating 15 minutes showed no variation in optical density.) The results are shown in Figure 2.

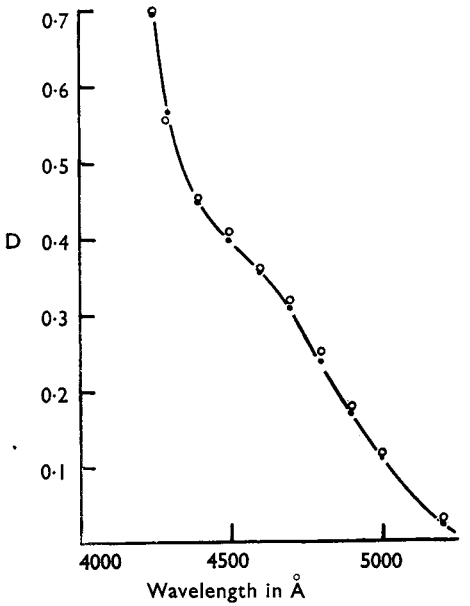

FIG. 1. Visible absorption spectrum of salicylaldehydesulphadimidine anil.
—·—·—·— 0·40 per cent. sulphadimidine, 1 cm. cell
o o o 0·10 per cent. sulphadimidine, 4 cm. cell.

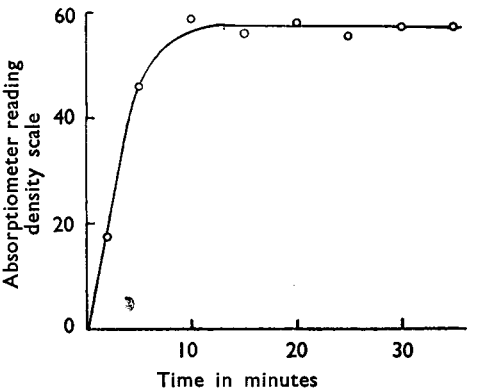

FIG. 2. Colour development time — Sulphadimidine with salicylaldehyde.

Salicylaldehyde required. Two solutions of salicylaldehyde in 95 per cent. ethanol, were made, 1 per cent. and 10 per cent. To a series of 10 ml. portions of a solution of 1 g. of sulphadimidine in 250 ml. of 95 per cent. ethanol were added successively increasing volumes of the above solutions, so as to provide from 0·01 g. to 1 g. of salicylaldehyde. Sufficient 95 per cent. ethanol was added to each so as to bring the volume to 20 ml. and the tubes were heated in a boiling water bath for 15 minutes. The tubes were removed and cooled, the volume again

adjusted to exactly 20 ml., and the absorption of the solutions measured against the blank solution. The results are shown in Figure 3.

It is evident that, for quantities up to 40 mg. of sulphadimidine, heating in ethanol with 1 g. of salicylaldehyde for 15 minutes ensures the complete development of the yellow colour.

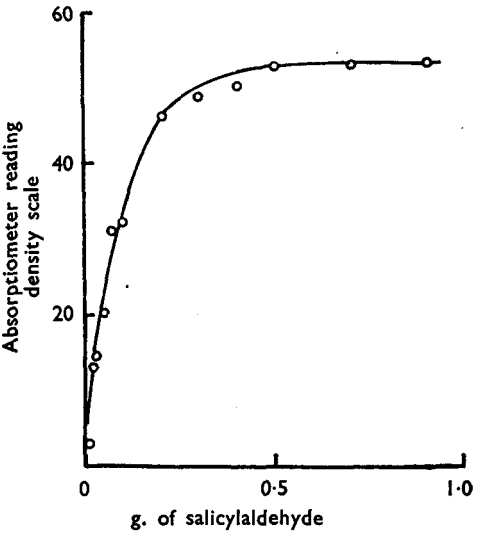

FIG. 3. Excess of salicylaldehyde required.

Production of calibration graph. A freshly made 0·4 per cent. solution of sulphadimidine in 95 per cent. ethanol was used for each run. (This solution, on standing, developed a yellow colour, and could not be used if kept for more than a few hours.) Volumes from 1 ml. to 10 ml. of this solution were introduced into boiling tubes and the volume adjusted to 10 ml. with 95 per cent. ethanol. 10 ml. of 10 per cent. solution of salicylaldehyde in 95 per cent. ethanol was added to each tube, and they were then heated in boiling water for 15 minutes. The tubes were removed, cooled and the contents transferred to 25-ml. graduated flasks. The volume was adjusted to 25 ml. with 95 per cent. ethanol and the optical densities of the solutions compared with a blank of equal volumes of the 10 per cent. ethanolic salicylaldehyde and 95 per cent. ethanol, using an Ilford 601 filter in the direct reading absorptiometer. The results (Table I) lacked the precision and reproducibility desired, and it was decided to continue the investigation using the Hilger "Spekker" absorptiometer, a

TABLE I

"OPTICAL DENSITIES" OF SULPHADIMIDINE-SALICYLALDEHYDE ANIL.
(Single cell absorptiometer—601 filter—1 cm. cell)

Volume of sulphadimidine solution, 0·4 g. per cent. w/v, ml.	Absorptiometer readings (Density scale)		
	1	2	3
1	6·3	5·3	4·0
2	12·0	13·1	9·0
3	16·8	18·7	14·5
4	21·0	22·1	19·2
5	26·0	25·8	23·8
6	30·9	31·2	28·2
7	35·6	36·3	32·5
8	39·0		
9	44·0	45·2	43·0
10	47·5	49·9	47·0

twin photocell "null" type of instrument. The general procedure for development of the colour was as above. A satisfactory calibration of high reproducibility was obtained (Fig. 4). For the blank the drum reading was set at 1·0.

Sulphadimidine was selected for these initial experiments on account of the solubility of its derivative with salicylaldehyde (see Table III).

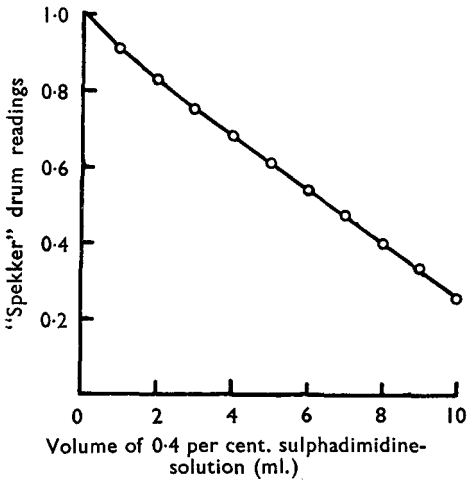

FIG. 4. Absorption of sulphadimidine-salicylaldehyde solutions.

(*f*) *Treatment of tablets.* To prepare a crystalline derivative a portion of the crushed tablet was boiled with 95 per cent. ethanol for a few minutes to extract the sulphonamide. To the filtered extract were added 5 drops of salicylaldehyde, the mixture again heated to the boil and set aside to cool, when the derivative separated. Alternatively the crushed tablet was boiled with 5 per cent. salicylaldehyde in 95 per cent. ethanol, the hot liquid filtered and set aside to cool. This method is preferred where the original sulphonamide is insufficiently soluble in boiling ethanol. At the time of publication no results are available for the quantitative examination of tablets.

RESULTS

(*a*) *Benzaldehyde derivatives.* In most cases a white or creamy white crystalline precipitate was readily obtained. The melting point of the product after recrystallisation did not in all cases agree with the original melting point. With some substances this divergence was so unusual as to suggest that the recrystallised material had a different identity (see Table II). The appearance under the microscope of each of the

TABLE II

BENZALDEHYDE DERIVATIVES OF THE "SULPHA" DRUGS

Derivative with	Melting point of original, °C.	Melting point after recrystallisation, °C.
Sulphanilamide	183 to 184	182 to 185
Sulphathiazole	203 to 204	203 to 204 (decomp.)
Sulphacetamide	99 to 101	148 to 150
Sulphaguanidine	208 to 210	209
Sulphapyridine	235 to 236	248
Sulphamethizole	158 to 160	117
Sulphamerazine	172 to 174	236
Sulphafurazole	192 to 193	189
Sulphadimidine	206 to 208	
Irgamid	212 to 213	

derivatives (not recrystallised) was, however, quite characteristic, and each type could be recognised at once without difficulty (Fig. 5). The dry products were quite odourless, insoluble or very slightly soluble in water, and soluble with decomposition in cold dilute acids to give a solution containing free benzaldehyde and a free aromatic NH_2– group. Determination of the nitrogen in certain of the products by the Kjeldahl method and by titration of the free NH_2– group produced on hydrolysis with dilute

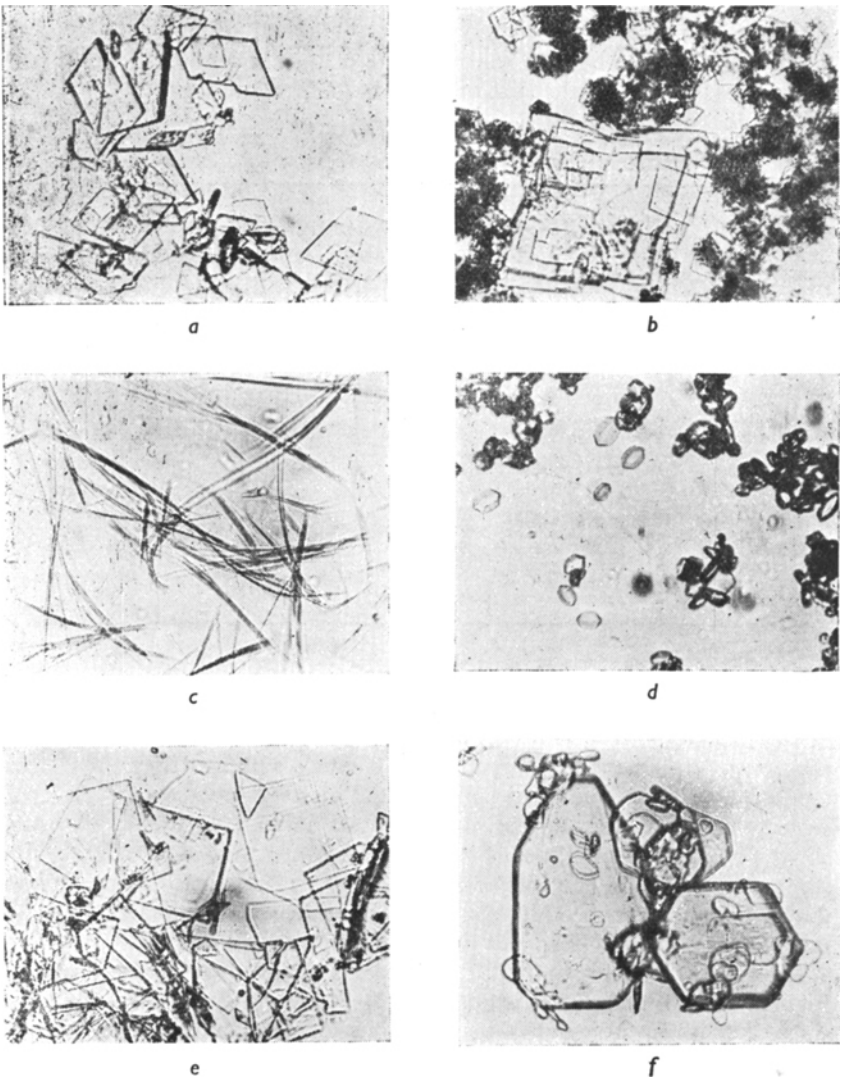

FIG. 5. Photomicrographs (× 200) of the benzaldehyde derivatives of (*a*) Sulphanilamide, (*b*) Sulphapyridine, (*c*) Sulphaguanidine, (*d*) Sulphathiazole, (*e*) Sulphamerazine and (*f*) Sulphacetamide.

acid suggested that these were not simple 1 : 1 molecular compounds and further investigation was deferred in favour of the more promising salicylaldehyde derivatives.

(*b*) *Salicylaldehyde derivatives.* With one exception the simple method described produced a coloured, crystalline precipitate. In the case of sulphadimidine a bright yellow solution, which failed to deposit crystals even on several days standing, was obtained. The solid was produced only by using much higher concentrations. The derivatives obtained by the method described were quite pure, and recrystallisation produced no significant change in the melting point. The full results are shown in Table III. It will be noted that the time of formation of the precipitate, and its general appearance are of value in its recognition.

TABLE III

SALICYLALDEHYDE DERIVATIVES OF "SULPHA" DRUGS

Derivative with	Appearance	Time taken to precipitate	Melting points		
			"Crude," ° C.	Recrystallised, ° C.	Others, ° C.
Sulphacetamide	Orange needles	30 minutes	217	215	212 to 214*a*
Sulphadimidine	Yellow powder	(Only from concentrated solution)		174	
Sulphaguanidine	Pale golden yellow	1 minute	248	247	225 to 226*a*
Sulphapyridine	Lemon yellow powder	2 minutes	244	245	241 to 242*a*
Sulphanilamide	Yellow needles	3 minutes	217 to 218	214 to 215	211*b*
Sulphathiazole	Pale orange-yellow prisms	1 minute	230	228 to 229*d*	215 to 217*a*
Sulphadiazine	Pale cream needles	15 minutes	258 to 259	258	244 to 245*a*
Sulphamerazine	Yellow clusters, and red needles (*c*)	2 minutes	235		225*a*
Irgafen	Bright golden yellow prisms	12 minutes	235	238	
Irgamid	Pale yellow needles	20 minutes	217	213	
Sulphafurazole	Light orange prisms	Several hours	192*d*		
Sulphamethizole	Orange needles	15 minutes	257		
Sulphasomidine	Pale yellow needles	Several hours	210		

a Castle, Witt and Poe[5]. *b* White, Witt, Biles and Poe[5]. *c* Changes entirely to the yellow form on drying at 100° C. *d* With decomposition.

The dry products were odourless and insoluble in water, but dissolved in aqueous alkali to give a bright yellow solution (due to the presence of the phenolic OH-group). From this alkaline solution they are reprecipitated by dilute acids and subsequently dissolve in excess of the acid, with some hydrolysis in the cold. Those derivatives which dissolved slightly in 50 per cent. aqueous ethanol were found to give complexes with some metals, notably copper and nickel. With ferric chloride an intense purple colour was produced. The formation of metal chelates with salicylaldehyde anils has been previously reported.[6]

(*c*) *Quantitative application.* The results are shown in Table I and Figures 1, 2, 3, and 4. It should be noted that in experiments with the single cell absorptiometer a scale reading of 100 represents an optical density of 1, and with the "Spekker" the optical density is represented by the difference between the drum reading and 1. (Strictly speaking, the term "optical density" can only be applied when monochromatic light is used.)

Discussion

The use of benzaldehyde in characterising the drugs of the sulphonamide group is of limited value. With some sulphonamides the melting point of the derivative is reproducible and in most cases microscopical examination of the crystals is a reliable indication of identity. The figures obtained for the determination of nitrogen (e.g., the benzaldehyde derivative of sulphaguanidine gave, by the Kjeldahl method and by titration with sodium nitrite of the aromatic $-NH_2$ group in the sulphaguanidine liberated on acid hydrolysis, the equivalent of about 23 per cent. of nitrogen) cannot be interpreted without further experimental work.

With salicylaldehyde, rapid and reliable confirmation of the identity of a sulphonamide is possible.

The quantitative determination can be carried out, in the case of sulphadimidine, in about 30 minutes. Quantities of the order of 20 mg. of this substance are determined with an estimated maximum error of 2 per cent. The probable error may well be much smaller than this, but no proper statistical examination has been carried out. There seems to be no reason why the method should not be suitable for the determination of tablets. One tablet provides sufficient material for both qualitative and quantitative investigation. The salicylaldehyde derivatives of some sulphonamides are sparingly soluble in ethanol, but the addition of ethylene glycol mono-ethyl ether to the solvent has been found to increase their solubility to an extent sufficient to permit measurement of absorption to be carried out. Other amino compounds show a similar behaviour with salicylaldehyde (for example benzocaine, orthocaine, procaine hydrochloride) and it is hoped to apply this quantitative determination to these substances, which are not, at present, subject to an official assay process. It is anticipated that some of the above topics will form the basis of subsequent communications.

Summary

1. A reaction between "Sulpha" drugs and certain aromatic aldehydes is described.

2. Salicylaldehyde gives characteristic derivatives with the majority of these drugs.

3. A method is described for the rapid determination of small amounts of sulphadimidine.

We wish to express our appreciation to the following firms, who supplied samples of pure materials:—Pharmaceutical Laboratories Geigy, Messrs. Sharp & Dohme, Roche Products, William R. Warner, and May and Baker.

References

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2. Castle, Witt and Poe, *J. Amer. chem. Soc.*, 1949, **71**, 228.
3. Castle, *J. Amer. pharm. Ass., Sci. Ed.*, 1951, **40**, 162.
4. Freeman, *Analyt. Chem.*, 1953, **25**, 1750.
5. White, Witt, Biles and Poe, *ibid.*, 1950, **22**, 950.
6. Martell and Calvin, *Chemistry of the Metal Chelate Compounds*, Prentice Hall, New York, 1952.

DISCUSSION

The paper was read by MR. P. H. B. INGLE.

MR. J. ISAACS (Dagenham) said that in the past *p*-dimethylaminobenzaldehyde had been used to determine amines. What, if any, were the advantages of salicylaldehyde over this reagent?

DR. F. HARTLEY (London) pointed out that the condensation between aromatic aldehydes and amines was a two-stage reaction. Anomalies found in the melting points of some of the benzaldehyde derivatives may have been due to the fact that the initial product was a straight addition product or at least only a partially dehydrated product which, through further heating and crystallisation, underwent the loss of the elements of water. In most cases the melting points after crystallisation appeared to confirm that probability. In the main they would expect the unsaturated product to have a higher melting point than the straight addition product. He was surprised at the results for sulphamethizole quoted in Table II. He wondered whether it was wise to judge the completion of the salicylaldehyde reaction simply by the yield of the product. The authors suggested that Castle's refluxing process did not appear to be essential for satisfactory yields. It would be helpful to know whether the melting point of the reaction product changed with time of refluxing. In Table III it appeared that the same product was being obtained by the reaction of salicylaldehyde with sulphacetamide, sulphanilamide and Irgamid. This suggested that in each case the anil corresponding to sulphanilamide was being obtained.

DR. G. FOSTER (Dartford) said that if some simple derivative, having a true melting point and not a decomposition point, could be formed by the aldehyde reaction, the work would be useful.

DR. J. W. FAIRBAIRN (London) asked whether the crystals always had the same shape or was there difficulty in reproducing them? Had the authors considered the use of optical crystallographic methods?

MR. R. F. TIMONEY (Dublin) said it seemed unusual to use benzaldehyde, the reactant, in the solvent for recrystallisation. Had they tried an inactive recrystallising agent, such as ligroin or light petroleum? Chromatography might be a method of obtaining pure material.

DR. A. H. BECKETT (London) said that elementary analysis should be carried out before statements were made about composition. Had the authors considered the possibility of isomerism?

MR. H. B. WOODHEAD (Manchester) said that the condensation products had been reported to occur in different crystalline forms with different melting points, and it would have been interesting had the authors used the Koeffler block as well as the standard method.

MR. P. B. H. INGLE, in reply, said that the benzaldehyde derivatives had been passed over in favour of the salicylaldehyde derivatives which were easier to prepare. He agreed it might be that they were getting, not a pure compound, but a mixture of primary addition compounds with

condensation products, which would explain the anomalous melting points. Certain of the benzaldehyde derivatives decomposed and others did not. No optical methods had been used in viewing the crystals. On the whole, the crystal form was the same every time they prepared these derivatives. Some of the benzaldehyde derivatives dissolved only slowly in ethanol, and a drop of benzaldehyde seemed to increase the solubility. Petroleum solvents were not found to be successful. Students using the method of preparation, which was designed for simplicity, had obtained melting points similar to those quoted. It might be that isomerism caused the differences in the melting point with the benzaldehyde derivatives, which was one of the reasons why they had been passed over.