Organic Mass Spectrometry, 1970, Vol. 4, pp. 299 to 313. Heyden & Son Limited. Printed in Northern Ireland

ELECTRON IMPACT STUDIES IN MEDICINE AND BIOCHEMISTRY—I:

A MASS SPECTROMETRIC STUDY OF THE IMPURITIES IN YELLOW PHENOLPHTHALEIN

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(*Received* 8 *June* 1970; *accepted* 8 *July* 1970)

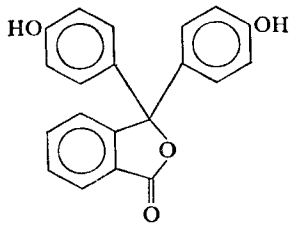
Abstract—A number of impurities have been extracted from yellow phenolphthalein by thin layer chromatography and characterized on the basis of their mass spectra. One of these by-products appears to be fluoran while the structure proposed for a second accounts for the properties ascribed to it by previous investigations. The bulk of the impurity appears to consist of condensation products of three molecules of phenol with two of phthalic anhydride.

INTRODUCTION

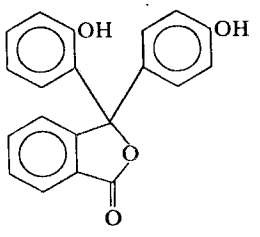
PHENOLPHTHALEIN (I) is available commercially in two forms, a white and a yellow. Of the two, the yellow material is more than twice as effective a laxitive as the white.[1] This has been attributed to the presence in the yellow compound of by-products of phenolphthalein sysnthesis one or more of which may have a higher potency than phenolphthalein itself. Alternatively an impurity, having no pharmacological action alone, may act as a promoter of phenolphthalein action.

Previous investigation[1] of yellow phenolphthalein established the presence of the following major by-products (Table 1) isophenolphthalein (II), fluoran (III) and an unknown substance denoted 'Compound V.'

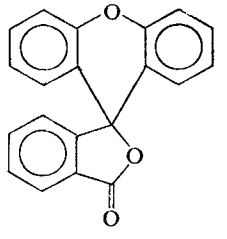
In the present paper some of the impurities in yellow phenolphthalein, after isolation by thin-layer chromatography, are further investigated by mass and infra-red spectrometry.



(I)



(II)



(III)

RESULTS

The thin layer chromatogram of the concentrated impurities obtained by recrystallization of yellow phenolphthalein is shown in Fig. 1 together with that of white phenolphthalein for comparison. The polarity of the fractions increases with decreasing R_f. The R_f of Fraction 3 and the colour developed on spraying were both

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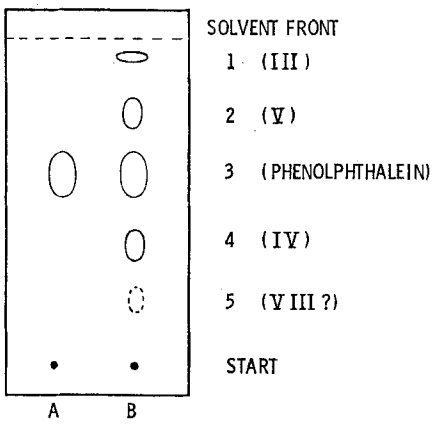


FIG. 1. Thin layer chromatograms of: (A) White phenolphthalein; (B) concentrated impurities from yellow phenolphthalein.

very similar to those of white phenolphthalein. This was subsequently confirmed by the identity of their mass and i.r. spectra.

Quantitative elution of each fraction, combined with the known weight of purified phenolphthalein obtained from the recrystallization enabled the purity of the yellow phenolphthalein specimen to be estimated. This is given in Table 1 together with those previously reported.[1] The present method does not distinguish between phenolphthalein and iso-phenolphthalein which are expressed as a combined percentage.

Examination of the mass spectra of fractions 1 to 4 over a wide evaporation temperature range indicated that each consisted very largely of a single specimen. In the following, the mass spectrum of pure phenolphthalein is discussed and the experience gained is used to interpret the spectra of the other fractions. The structures postulated for the fragment ions in the following Schemes must, in absence of isotopic labelling studies, be somewhat speculative. They have been chosen as the most probable on the basis of maximum resonance stabilization and minimum number of unpaired electrons. Where appropriate, the mass spectral observations are supplemented by i.r. spectral data.

TABLE 1. COMPOUNDS ISOLATED FROM YELLOW PHENOLPHTHALEIN

	Present study	Hubacher[1]		
		A	B	C
Phenolphthalein }	%	%	%	%
Iso Phenolphthalein }	97·5	94·8	95·0	93·2
Fluoran	0·2	0·2	0·3	0·3
Phenolphthalin	—	0·9	0·9	—
2-(4-hydroxy benzoyl) benzoic acid	—	(0·005)	—	0·1
Structure V (Mol. wt. 524)	0·7	—	0·06	0·04
Structure IV (Mol. wt. 542)	0·6	—	—	—
Structures VI to VIII (Mol. wt. 730 to 766)	(0·005)	—	—	—
Recovery	99·0	95·9	96·3	93·7

White phenolphthalein (also fraction 3)

The i.r. spectrum was characteristic of Structure I, with prominent bands at 3300 to 3400 cm^{-1} (OH hydrogen bonded) 1740 cm^{-1} (C=O, γ-lactone) together with a large number of bands characteristic of the aromatic nuclei.[2]

The mass spectra of white phenolphthalein and its dimethyl ether are shown in Figs. 2 and 3. Metastable ions observed in the phenolphthalein spectrum are listed in Table 2.

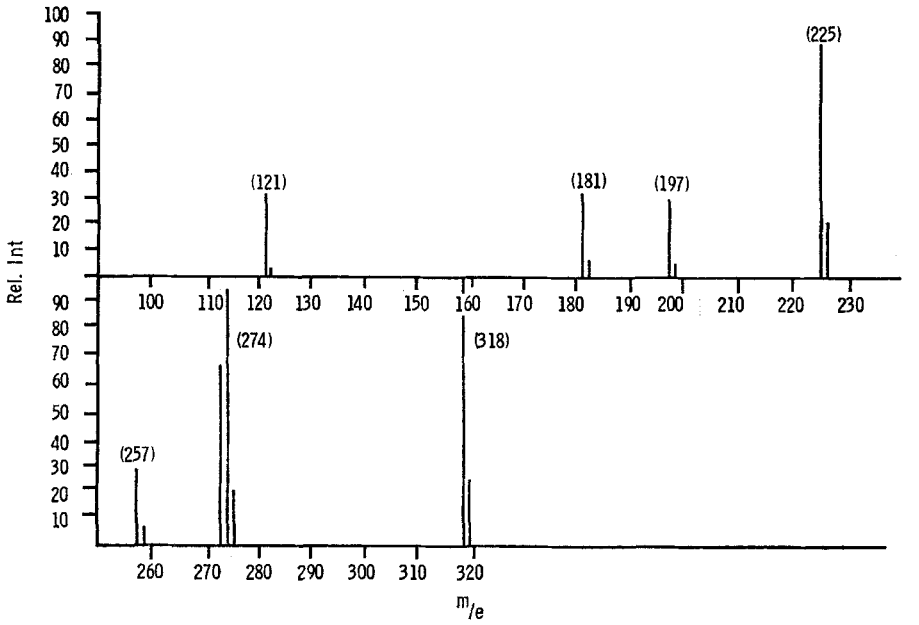


FIG. 2. Mass spectrum of 'White' phenolphthalein.

Since the Mattauch–Herzog analyzer of the SM1 mass spectrometer severely discriminates against metastable ions, they are only observed with abnormally large samples. In most cases apart from phenolphthalein there was insufficient material for metastable ions to be detected under normal instrument conditions.

There are two primary decomposition paths for the phenolphthalein molecule ion. One involves the loss of a hydroxy-phenyl group to give a conjugated ion of *m/e* 225. This ion can decompose further with loss of CO or CO_2 to give ions of *m/e* 197 and *m/e* 181. Cleavage of C_6H_4 from *m/e* 197 probably gives rise to $HOC_6H_4C{\equiv}O^+$ at *m/e* 121. The second path involves elimination of CO_2 from the lactone ring to give a resonance stabilized ion *m/e* 274. This ion then loses OH to give an *m/e* 257 fragment. Straightforward cleavage would result in a bi-radical ion but a 1:3 hydrogen atom rearrangement would allow the formation of a more stable even-electron structure. These observations are summarized in Scheme 1. The fragmentation of the dimethyl ether appears to take place in a similar manner. For each fragment in the phenolphthalein spectrum, there appears to be a corresponding fragment shifted in mass by 14 or 28 a.m.u., as appropriate, in the dimethyl phenolphthalein spectrum. These are also indicated in Scheme 1.

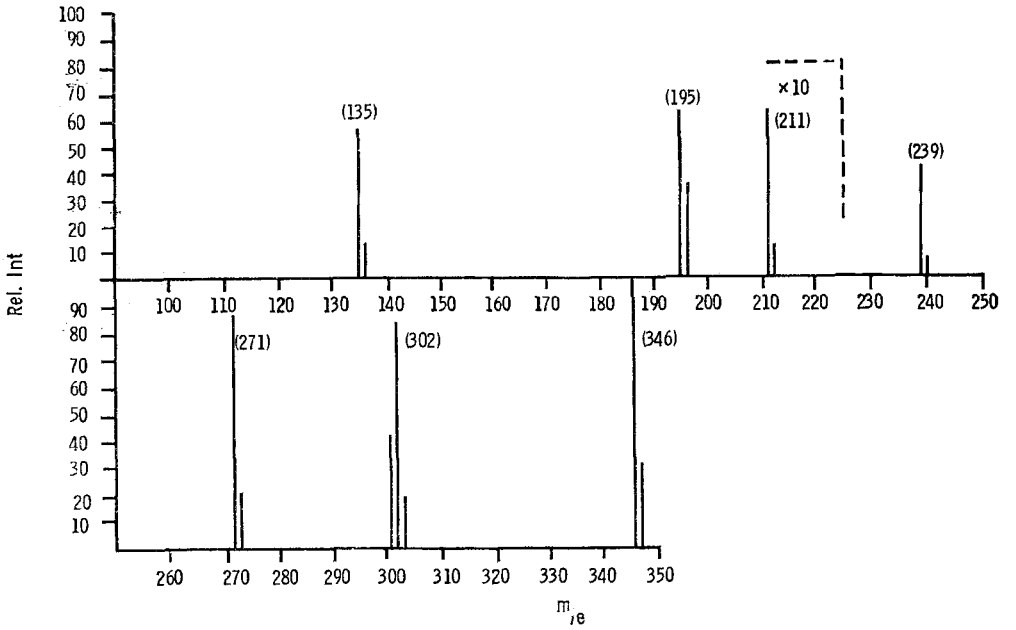


FIG. 3. Mass spectrum of dimethyl phenolphthalein.

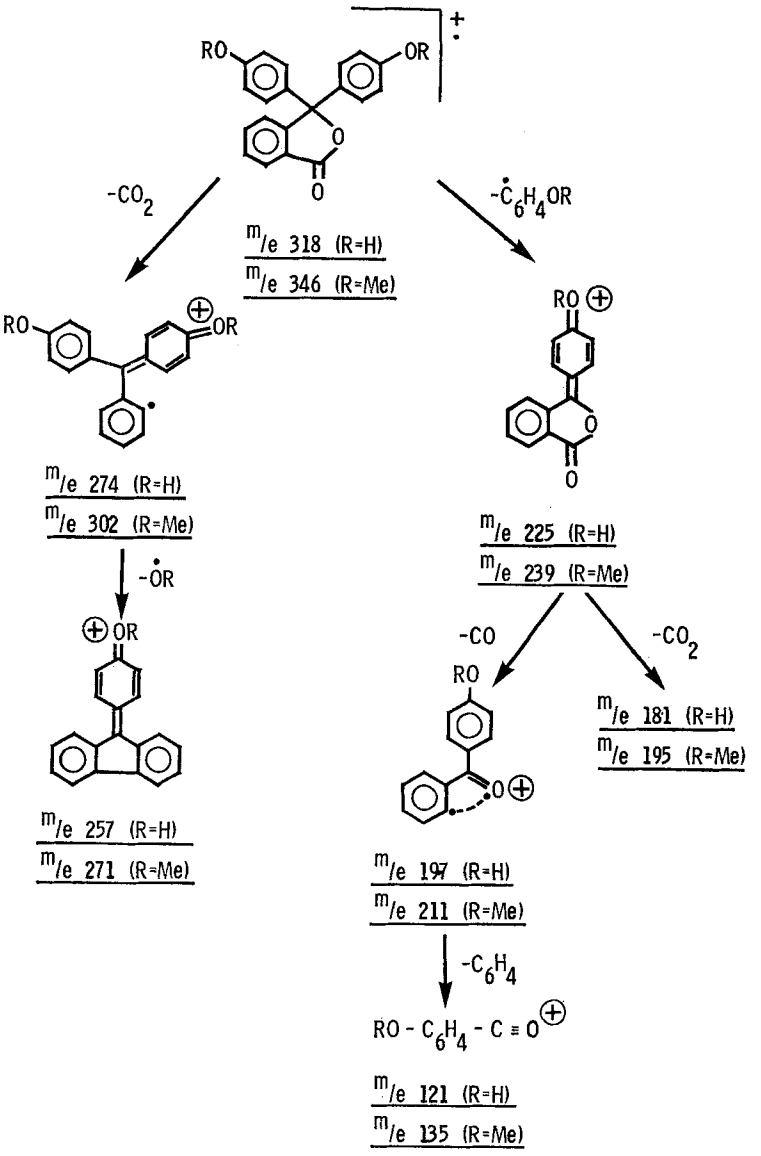
Fraction 1

This substance was strongly fluorescent in the u.v. but did not give a colour with K_2CO_3 solution. Its i.r. spectrum was of low intensity due to the small sample size. Only the γ-lactone carbonyl band at 1760 cm^{-1} could be reliably assigned. OH appeared to be absent.

The mass spectrum of fraction 1 is shown in Fig. 4. The molecular ion $[M]^{+\cdot}$ has a mass of 300·079 corresponding to an atomic composition $C_{20}H_{12}O_3$. Prominent fragments are observed at 256 $[M - CO_2]^{+\cdot}$, 224 $[M - C_6H_4]^{+\cdot}$, 196 and 168. The lack of OH absorption in the i.r. coupled with the high R_f of the compound indicate that phenolic and carboxyl groups are absent. This is confirmed by the failure of fraction 1 to form a methyl derivative. The substance does appear, however, to have a lactone ring. The known by-product fluoran (III) could give rise to the observed spectrum as shown in Scheme 2.

TABLE 2. METASTABLE IONS IN THE SPECTRUM OF PHENOLPHTHALEIN

Observed Mass	Assignment	Theoretical Mass
241·1	$274^+ \rightarrow 257^+ + 17$	241·05
236·1	$318^+ \rightarrow 274^+ + 44$	236·09
172·5	$225^+ \rightarrow 197^+ + 28$	172·48
159·2	$318^+ \rightarrow 225^+ + 93$	159·20
145·6	$225^+ \rightarrow 181^+ + 44$	145·60



SCHEME 1

Fraction 2

This material was also strongly fluorescent in u.v. light, appeared yellow in white light and gave a deep yellow colour on spraying. Its i.r. spectrum was very similar to that of phenolphthalein and showed OH, γ-lactone and prominent *o*-disubstituted benzene ring bands. COOH and COO^- were not detected.

The mass spectrum of fraction 2 is shown in Fig. 5. The molecule-ion at *m/e* 524·125 has the composition $C_{34}H_{20}O_6$. Fragments at *m/e* 480 and 435 indicate stepwise loss of two molecules of CO_2, the second with concurrent loss of one H atom.

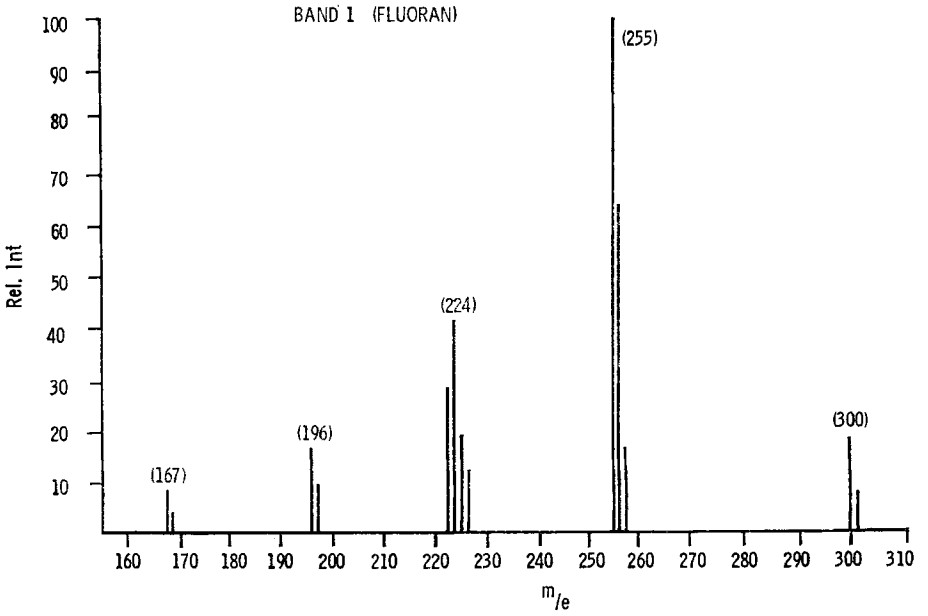
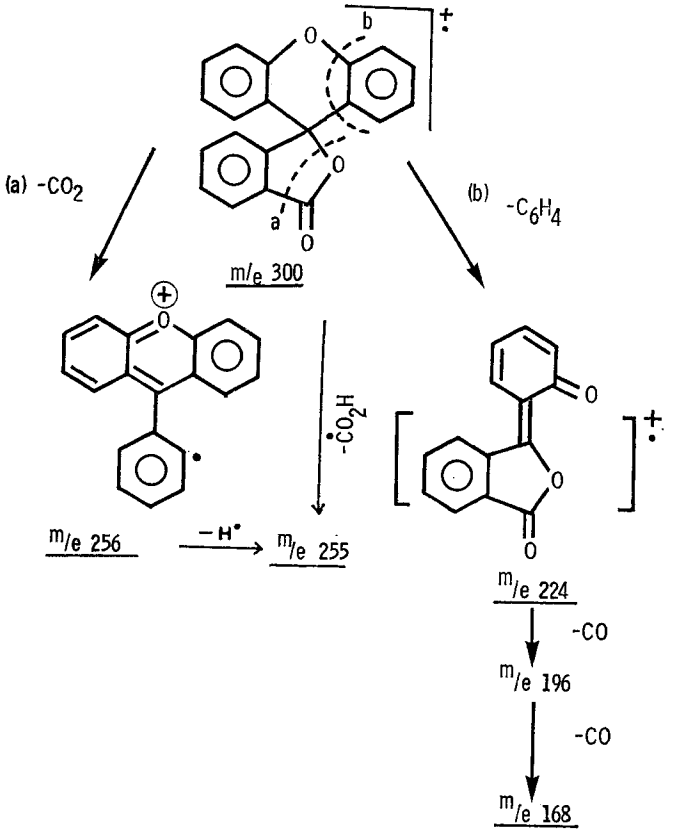


FIG. 4. Mass spectrum of t.l.c. fraction 1.



SCHEME 2

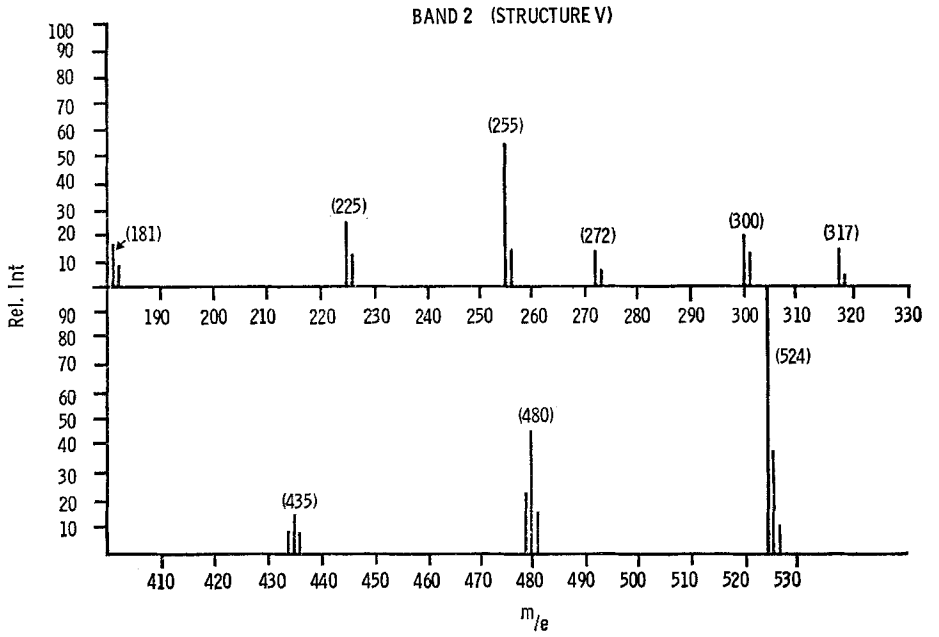
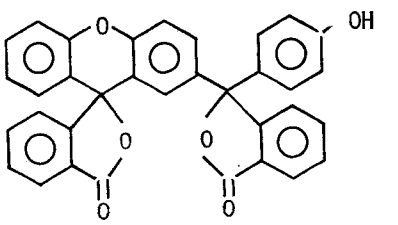


FIG. 5. Mass spectrum of t.l.c. fraction 2.

Since COOH is known to be absent, it is likely that two lactone rings are present. The lower mass fragments fall into two groups: (a) *m/e* 300 and 255 as observed for fluoran and (b) *m/e* 317, 272, 225 and 181 which are indicative of the fragmentation of a phenolphthaleinyl residue. Thus it appears that the molecule combines the structures of both fluoran and phenolphthalein.

Methylation (see Fig. 6) increases the molecular ion mass by 14 a.m.u. indicating one phenolic OH group. The fragments *m/e* 480 and 435 are also shifted to *m/e* 494 and 449 respectively. The fragments at *m/e* 300 and 255 which appear to be derived from the fluoran portion of the molecule remain unchanged on methylation but those thought to be derived from the phenolphthalein moiety (181, 225, 272 and 317) are all increased by 14 m.u. These results can be interpreted on the basis of structure V according to Scheme 3.



V

Fraction 4

This material was faintly fluorescent on u.v. irradiation, was invisible in white light and stained a deep purple on spraying. The i.r. spectrum was very similar to that of

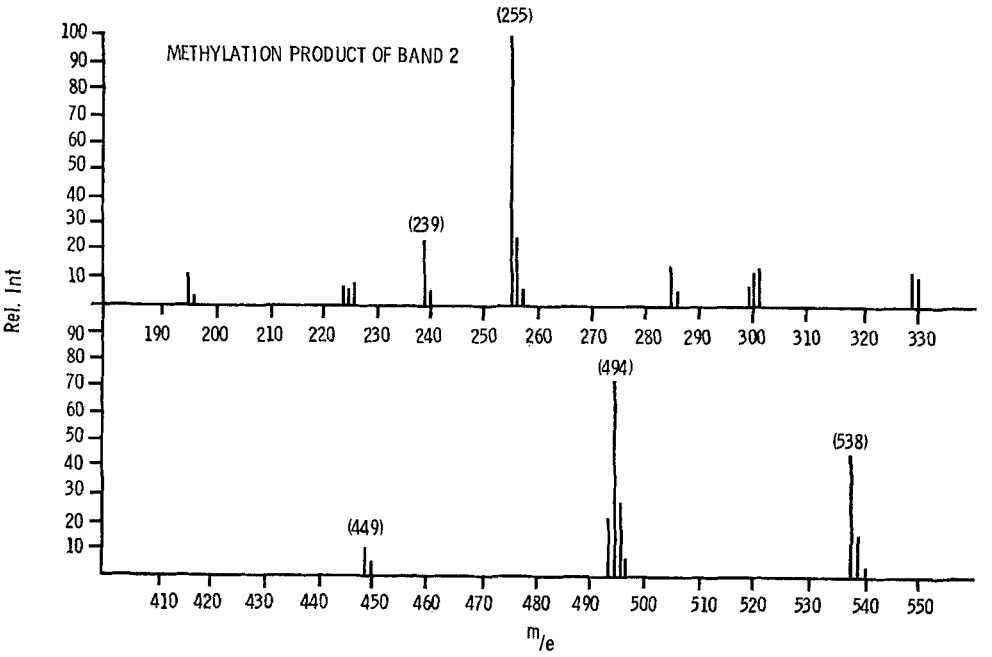


FIG. 6. Mass spectrum of the permethylation product of fraction 2.

phenolphthalein showing prominent OH, γ-lactone and aromatic nucleii bands but no COOH or COO^-.

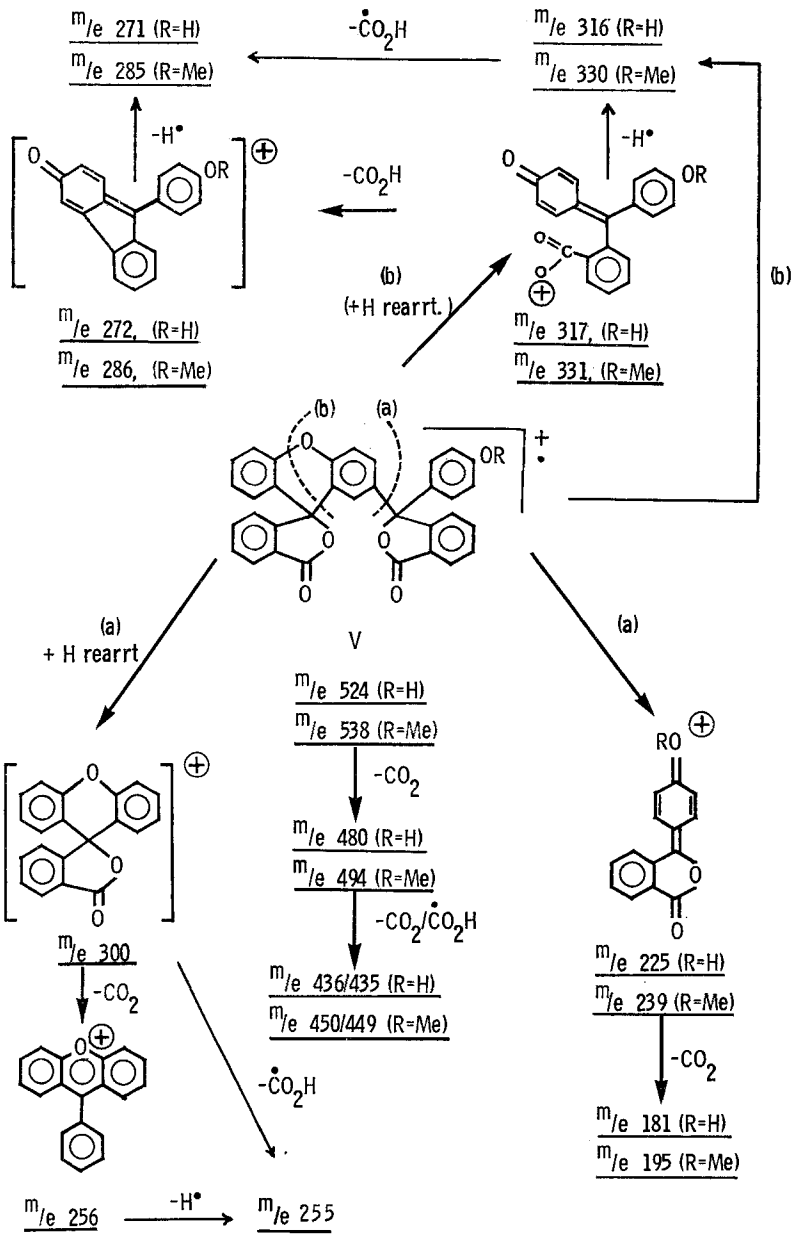
The mass spectrum of fraction 4 is shown in Fig. 7. The molecular ion (*m/e* 542·136) has the composition $C_{34}H_{22}O_7$ corresponding to an increment of H_2O over the composition of structure V. Two molecules of CO_2 are lost in turn giving fragments of *m/e* 497 and 453, one step involving simultaneous loss of an H atom. The remaining fragments from 318 to 181 bear a strong resemblance to those derived from the fragmentation of phenolphthalein. There is no evidence of any fragments arising from a fluoran residue.

Permethylation leads to the formation of a trimethyl derivative whose spectrum is shown in Fig. 8. The decomposition of the ether is somewhat more complex than that of the free phenol. The masses of the fragments arising from loss of CO_2 are both increased by 42 m.u, however, while intense fragments are observed at *m/e* 301, 270 and 239 arising from the dissociation of a dimethyl phenolphthaleinyl residue. Other fragmentation paths arise from fragments of *m/e* 526 $[M - CO_2 - CH_2]^+$ and *m/e* 509 $[M - CO_2 - CH_3O]^+$. The former was originally suspected to arise from loss of CO_2 from a dimethyl derivative of fraction 4. No molecule ion corresponding to such a compound could be detected at *m/e* 570, however, hence *m/e* 526 appears to be a genuine fragment of *m/e* 584.

The foregoing observations can be accommodated in structure IV and the mass spectra of IV and its trimethyl ether rationalized according to Schemes 4 and 5.

Fraction 5

This was not present in sufficient quantity to be identified separately. Its lower R_f indicated greater polarity than compounds I to V. When the mass spectrum of the



SCHEME 3

total concentrated impurities was obtained before t.l.c. separation, substances of molecular weights 730, 748 and 766 were detected in trace amounts. Their partial spectrum is shown in Fig. 9. Identification can only be very speculative but they appear to be condensation products of four molecules of phenol with three of phthalic anhydride. By analogy with IV and V, the structures VI to VIII are suggested for

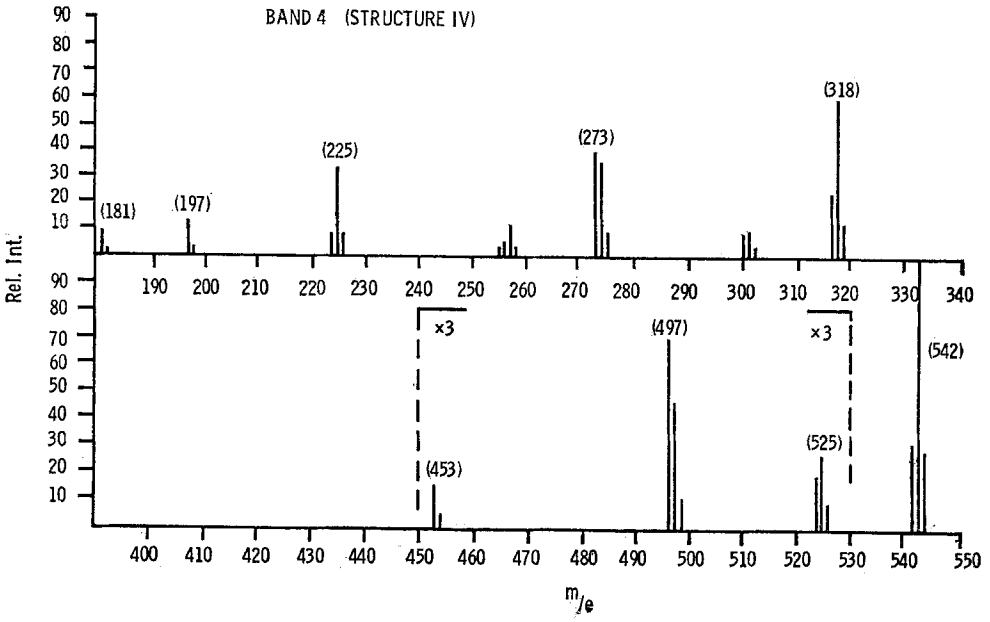


FIG. 7. Mass spectrum of t.l.c. fraction 4.

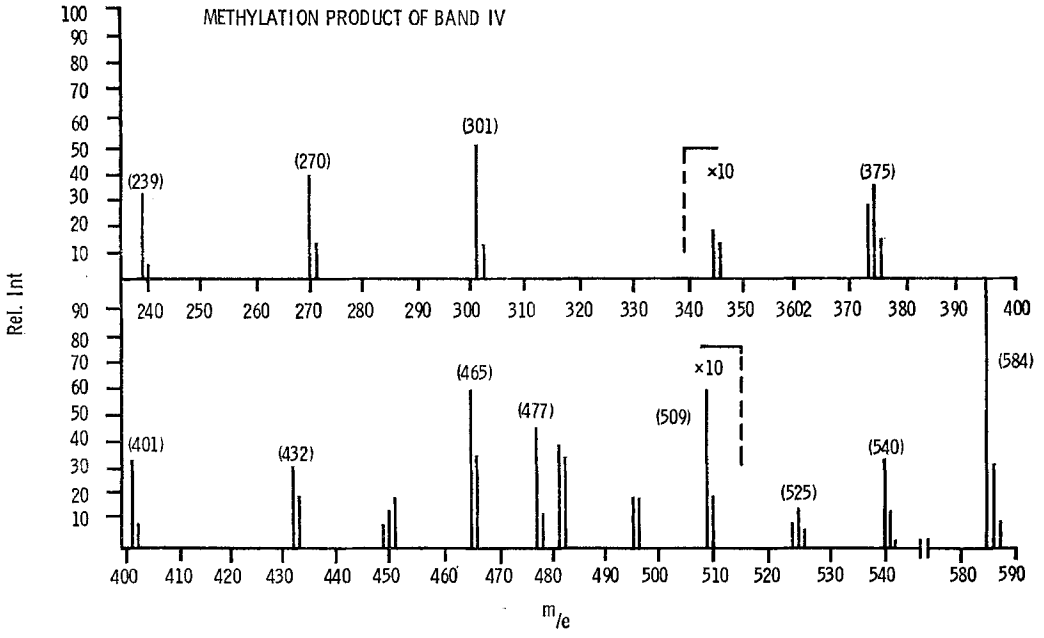
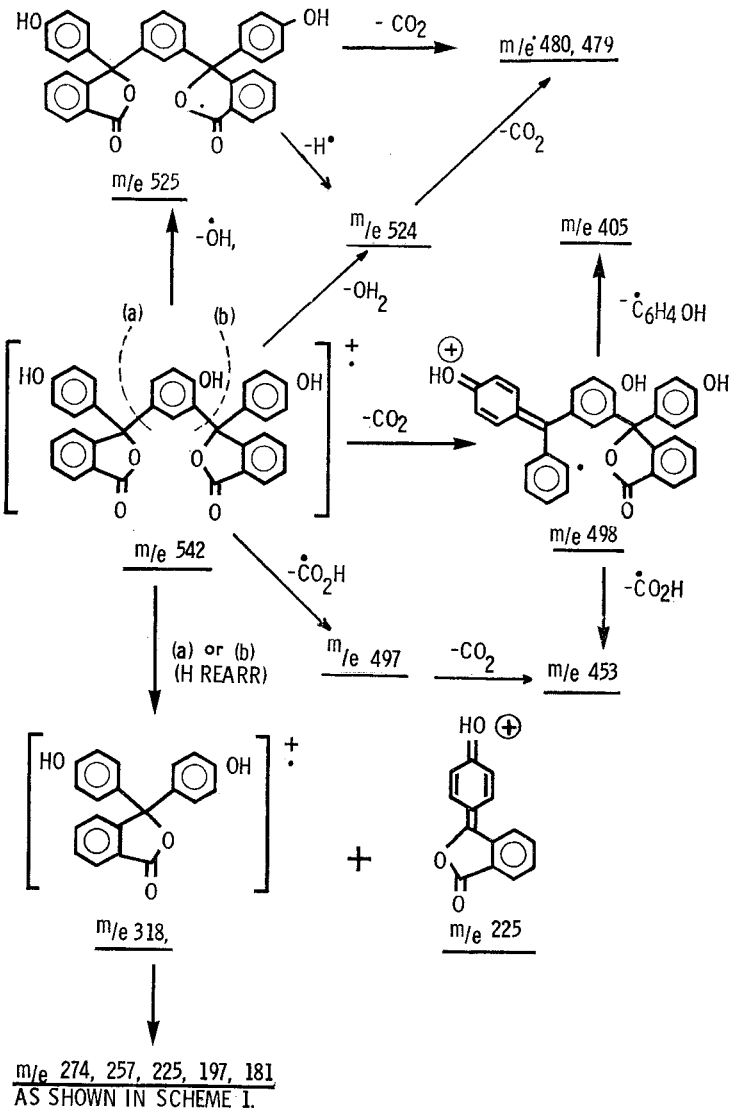
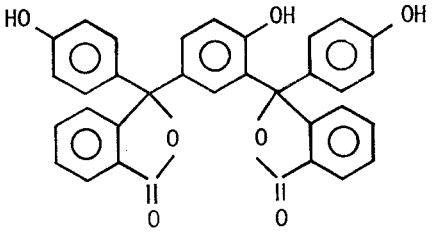


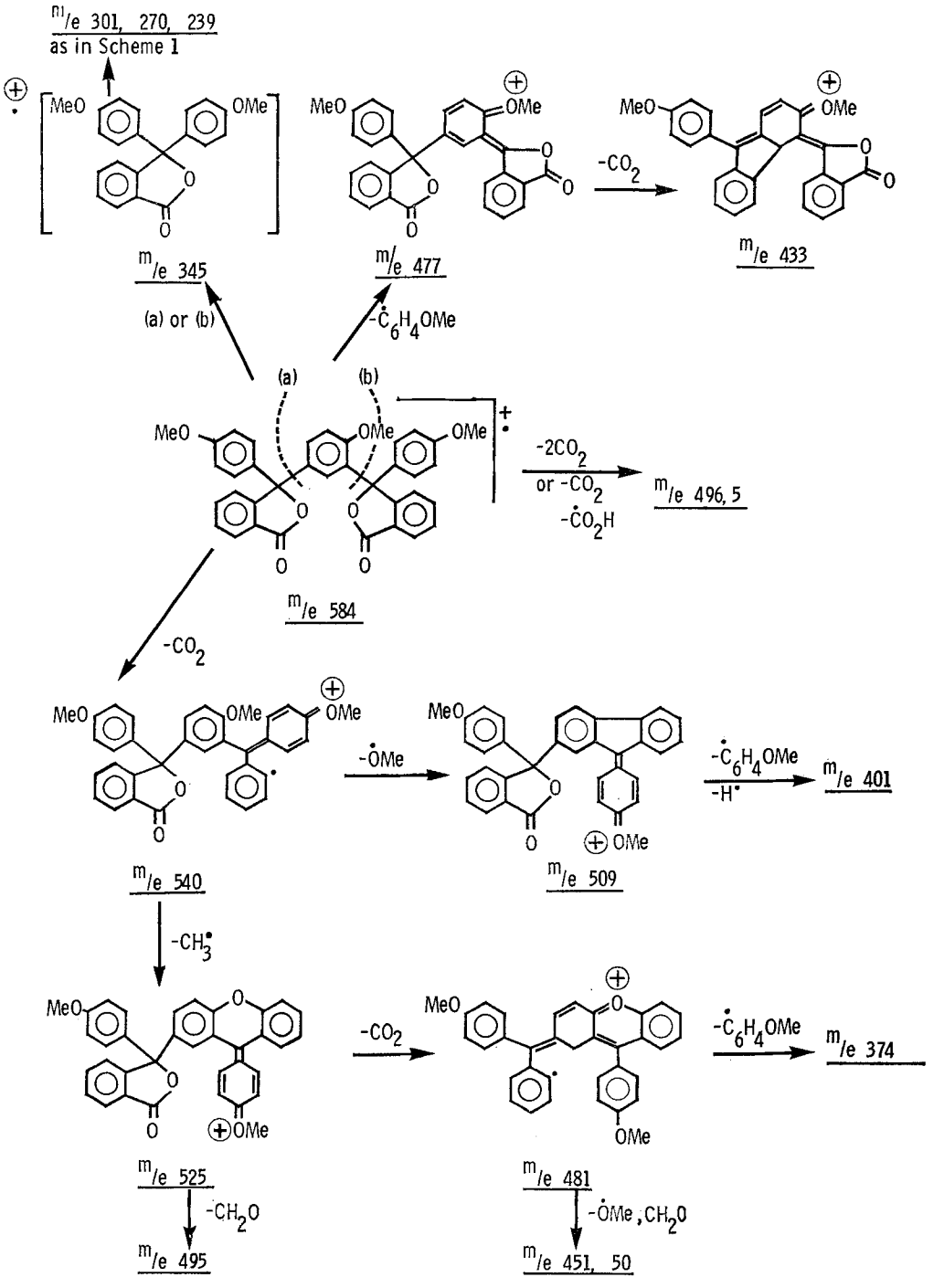
FIG. 8. Mass spectrum of the permethylation product of fraction 4.


HO
OH
- CO2
m/e 480, 479
-H•
-CO2
m/e 525
m/e 524
m/e 405
-OH,
-OH2
-C6H4 OH
(a)
(b)
HO
OH
OH
-CO2
m/e 542
m/e 498
-CO2H
-CO2H
(a) or (b)
(H REARR)
m/e 497
-CO2
m/e 453
m/e 318,
m/e 225
m/e 274, 257, 225, 197, 181
AS SHOWN IN SCHEME 1.

SCHEME 4



IV


m/e 301, 270, 239
as in Scheme 1
MeO
OMe
m/e 345
m/e 477
m/e 433
-CO2
(a) or (b)
-C6H4OMe
(a)
(b)
-2CO2
or -CO2
-CO2H
m/e 496, 5
m/e 584
-CO2
-OMe
-C6H4OMe
-H•
m/e 401
m/e 540
m/e 509
-CH3•
-CO2
-C6H4OMe
m/e 374
m/e 525
-CH2O
m/e 495
m/e 481
-OMe, CH2O
m/e 451, 50

SCHEME 5

these. Since VIII has four hydroxyl groups, its R_f would be less than that of IV and hence VIII may be a partial constituent of fraction 5. The low concentration of VI to VIII, estimated as 0·005% of the total yellow phenolphthalein concentration, would make their detection on the thin layer chromatogram difficult. This would be especially true of VI and VII whose R_f values would probably be too close to fluoran and phenolphthalein respectively for adequate separation.

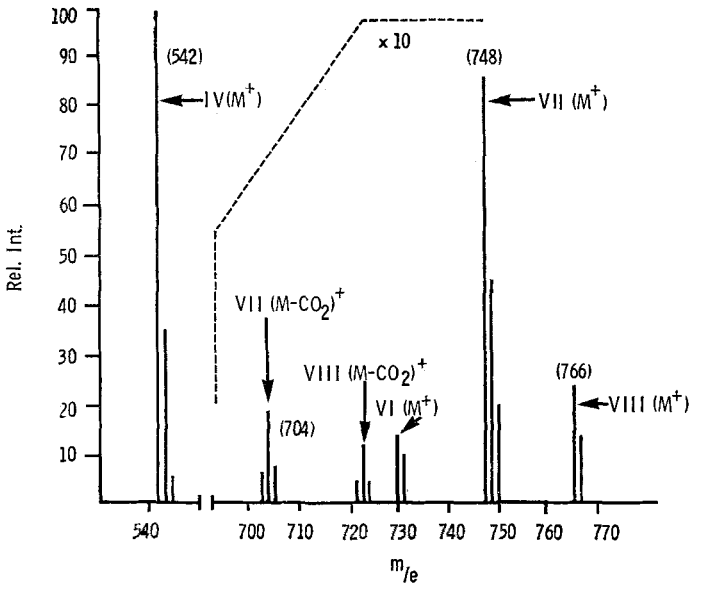
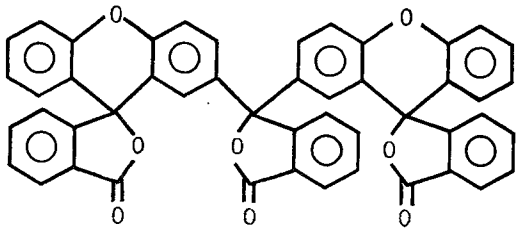


FIG. 9. Mass spectrum of the residues from the recrystallization of yellow phenolphthalein.

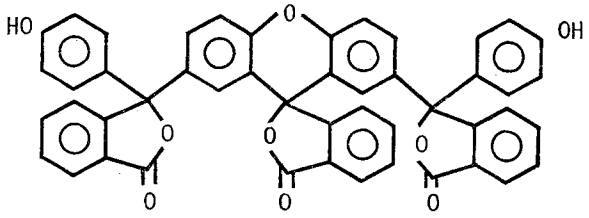
DISCUSSION

From Table 1 it is evident that the purity of the yellow phenolphthalein determined here is in reasonable agreement with previous observations. Hubacher[1] has shown that the nature of the impurities varies considerably with the manufacturer. In the present specimen, no clear evidence of phenolphthalin or 2-(4-hydroxy benzoyl) benzoic acid was obtained. On the other hand neither Compound IV of molecular weight 542, nor the higher condensation products VI to VIII, appear to have been described previously. The proposed structure V accounts for the properties of 'Compound V' described by Hubacher who gave its molecular weight as 524 and showed it to contain two lactone rings and one phenolic OH group.

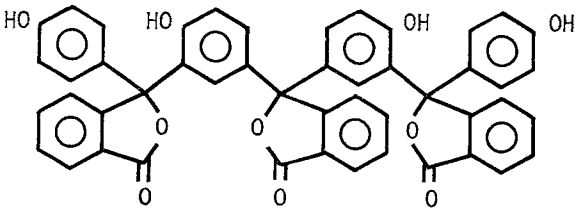
Yellow and white phenolphthalein are prepared in the same way by reaction of phenol with phthalic anhydride in the presence of a dehydrating catalyst. The white material is obtained from the crude product by recrystallization from alcohol; the yellow is reprecipitated from a solution of the crude in alkali by dilute acid. Compounds IV, V, VII and VIII could arise as by-products of this synthesis. Since they all contain phenolic OH groups they would be soluble in alkali and be reprecipitated along with the phenolphthalein. Fluoran and compound VI have no phenolic OH and



VI

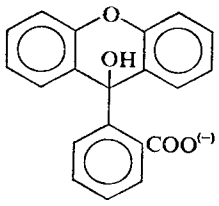


VII



VIII

should be insoluble in alkali. Small amounts may dissolve in the form of their hydrolysis products, however (e.g. IX for fluoran) which would recyclize to the lactone on acidification.



(IX)

Fluoran could also be formed as a thermal decompositon product of V but this seems less likely since the fluoran would probably be accompanied by an approximately equivalent amount of 2-(4-hydroxyl benzoyl) benzoic acid which was not detected in this study.

EXPERIMENTAL

20 g of yellow phenolphthalein were recrystallized from about 100 ml of ethanol. The precipitate of white phenolphthalein was filtered and washed with cold ethanol. The resulting supernatant,

containing the coloured impurities was concentrated and a further precipitate of white phenolphthalein filtered off. The resulting dark brown solution was concentrated to about 1 ml and chromatographed on 20 × 20 cm plates coated with silica gel (Mercke). Satisfactory separation was achieved using the solvent system benzene: methanol: acetic acid (45:8:4). The fractions were visualized by their u.v. fluorescence and by spraying a portion of the plate with N—K_2CO_3 solution followed by heating to develop the colours. Each fraction was then isolated for spectroscopic examination by scraping the unsprayed silica gel from the appropriate part of the plate and eluting with methanol. An approximate quantitative estimate of each impurity was obtained from the weights of the residues after evaporation of the solvent from the eluates and the weight of white phenolphthalein obtained at recrystallization.

I.r. spectra were obtained as KBr discs on a Perkin–Elmer model 237 spectrophotometer. Mass spectra were obtained on a Varian MAT model SM1 mass spectrometer. Samples were admitted by the direct insertion technique. The running conditions were ionizing current—300 μA, ionizing voltage—70 eV, source temperature 200°C. Spectra of each fraction were obtained at a series of evaporation temperatures from 50°C to 350°C in order to check whether any of the t.l.c. fractions were mixtures. Atomic compositions of the molecular ions were determined by accurate mass measurements. The difference between the measured and theoretical mass in each case was less than the precision of the measurement ($\pm$1 millimass unit).

Mass spectra were also obtained of the methyl derivatives of fractions 1 to 4. They were prepared by reaction with freshly prepared diazomethane in ether followed by evaporation of excess reagent after allowing to stand for about 10 mins. at room temperature. Mass spectra showed that under these conditions, only the phenolic groups were methylated and no methylene insertion into the lactone ring took place.

Acknowledgements—We are indebted to Professor E. F. Scowen for his interest and the Board of Governors of St. Bartholomews Hospital for their generous research grants. The mass spectrometer was purchased with the help of a grant from the Fleming Memorial Trust.

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