THE ASSAY OF PHENYLINDANEDIONE

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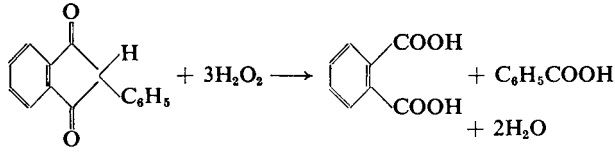
The only assay of phenylindanedione (phenindione) which appears in the literature is a spectrophotometric one published by the Council of Pharmacy and Chemistry of the American Medical Association[1], and a content of not less than 95·0 per cent. and not more than 105·0 per cent. of $C_{15}H_{10}O_2$ is required. These limits appear to be rather wide for what should be an entity and an assay lending itself to greater precision than is usually obtainable spectrophotometrically seems desirable.

Gravimetric method

Phenylindanedione reacts readily with 2:4-dinitrophenylhydrazine but, owing to the great degree of enolisation (and possibly also steric hindrance), it was not found possible to prepare the bis-dinitrophenylhydrazone or, in fact, to obtain reproducible figures by gravimetric methods. Various modifications of the procedures used by the author for the gravimetric estimation of vanillin[2] yielded results varying from 63·9 to 110·7 per cent. calculated with reference to the monodinitrophenylhydrazone.

Oxidation method

A method which seemed to show more promise was the titration of the acids produced by oxidation of the diketone with alkaline peroxide solution. It seemed a reasonable assumption that one molecule of phenylindanedione would be quantitatively oxidised to one molecule each of phthalic and benzoic acids. 20 volume hydrogen peroxide in the presence of N sodium hydroxide as used in the assay of vanillin[2] gave incomplete oxidation as evidenced by the persistence of a yellow colour, but stronger peroxide gave a colourless solution containing 100 per cent. of the theoretical amount of benzoic acid; the phthalic acid was obtained in only 75 per cent. yield, due to further oxidation, as evidenced by the high titre and presence of carbonate in the solution. According to the following equation 1 ml. of 0·5N hydrochloric acid ≡ 0·03703 g. of $C_{15}H_{10}O_2$ but,



owing to the partial oxidation of the phthalic acid, this figure yields results over 10 per cent. too high. However, providing the weight of sample taken does not exceed 0·45 g. consistent results are obtained and

TABLE I

THE ESTIMATION OF PHENYLINDANEDIONE BY OXIDISING WITH ALKALINE PEROXIDE SOLUTION AND BACK-TITRATING THE EXCESS OF ALKALI

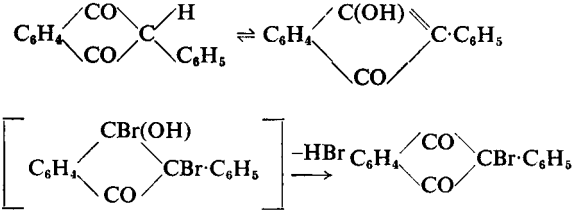
Sample mg.	0·5N alkali used by acids from 1 g. of material ml.	$C_{15}H_{10}O_2$ per cent.
475·4	29·85	100·0*
312·4	29·82	99·9
286·0	29·66	99·4
309·4	29·82	99·9
382·5	29·75	99·7
524·5	30·67	102·7
749·2	32·82	110·0
771·5	31·82	106·6

* Figure obtained by using the factor "each ml. of 0·5N hydrochloric acid is equivalent to 0·03350 g. of $C_{15}H_{10}O_2$."

the method is quite applicable if the arbitrary factor, "1 ml. of 0·5N hydrochloric acid $\equiv$ 0·03350 g. of $C_{15}H_{10}O_2$" is employed (see Table I).

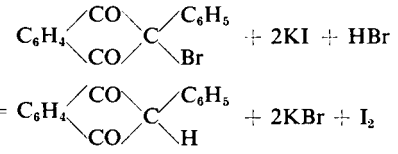
Bromination method

The presence of a $>$CH-grouping between two carbonyl groupings led to the suggestion that phenylindanedione could be quantitatively brominated. Owing to the lability to potassium iodide of the bromine atom in the –CO : CBr·Ph·CO– group, the estimation of excess of standard bromine was not possible, but after the removal of the excess of bromine with β-naphthol the addition of potassium iodide to the bromoketone reconverted the latter to phenylindanedione with the liberation of two equivalents of iodine which could be titrated with thiosulphate. This was the method employed by Kurt Meyer[3] for the determination of the percentage of enol form present in keto-enol tautomeric mixtures such as aceto-acetic ester. It would thus seem that the time of bromination must be sufficient to enable the conversion of the keto form to the enol form and its subsequent bromination to be completed, according to the following equations.



Surprisingly enough the results show that even after allowing bromination to proceed for only 5 seconds over 99 per cent. of bromoketone is produced. This indicates that either (*a*) the diketone exists entirely in the enolic form in alcoholic solution or (*b*) the rate of enolisation in the presence of bromine is extremely rapid. For the most consistent results it is suggested that bromination be allowed to proceed for 5 minutes; 15 minutes bromination yields slightly high figures (see Table II).

The action of potassium iodide on the bromo-ketone is expressed by the following equation.



It has been found unnecessary to add mineral acid with the potassium iodide as the ethanolic solution already contains one equivalent of hydrobromic acid liberated during the bromination. Good results were obtained for all weights of sample between 150 mg. and 420 mg.

TABLE II

THE ESTIMATION OF PHENYLINDANEDIONE BY THE BROMINATION METHOD

Sample mg.	Time of bromination	$C_{15}H_{10}O_2$ per cent.
362·9	5 seconds	99·65
282·9	1 minute	99·68
198·0	2 minutes	99·75
419·6	2 "	100·2
282·9	5 "	99·93
362·9	5 "	100·0
419·6	5 "	100·3
419·6	5 "	100·0
362·9	10 "	100·5
257·6	15 "	100·2
219·2	15 "	101·2
153·6	15 "	100·4
254·4	15 "	99·84
362·9	20 "	100·4

Spectrophotometric method

As the usual weight of phenylindanedione in tablets is 50 mg., an assay requiring a smaller quantity of material than about 300 mg. is not normally necessary as even this quantity represents only 6 tablets. However, certain tablet excipients would interfere with the alkaline peroxide method and might also render worthless any results obtained by the bromination method, although the rapid addition of bromine and its equally rapid removal with potassium iodide is fairly specific to ketones possessing α hydrogen atoms. Nevertheless, it seemed desirable to examine the curves obtained by means of the ultra-violet spectrophotometer from solutions made with different solvents as the degree of enolisation, and hence $\lambda_{max.}$ and $E_{max.}$ would be expected to vary widely with the nature of solvent, *p*H, etc. The solvents chosen were I, ethanol (95 per cent.); II, *cyclo*hexane (in which enolisation would probably be at a minimum); III, N hydrochloric acid in ethanol (in which ionisation of the enolic form and hence enolisation would be suppressed); and IV, 0·1N sodium hydroxide in which enolisation and ionisation would be expected to be complete. The last of these yields the most prominent peak and being virtually a buffered solution is less likely to variation in $E_{max.}$ due to slight changes of *p*H. The 4 curves are shown in Figure 1 and the values for the different maxima are given in Table III.

From the curves (and the observed colours of the solutions) it is clear that the ketone is largely enolised in ethanolic solution (borne out by the

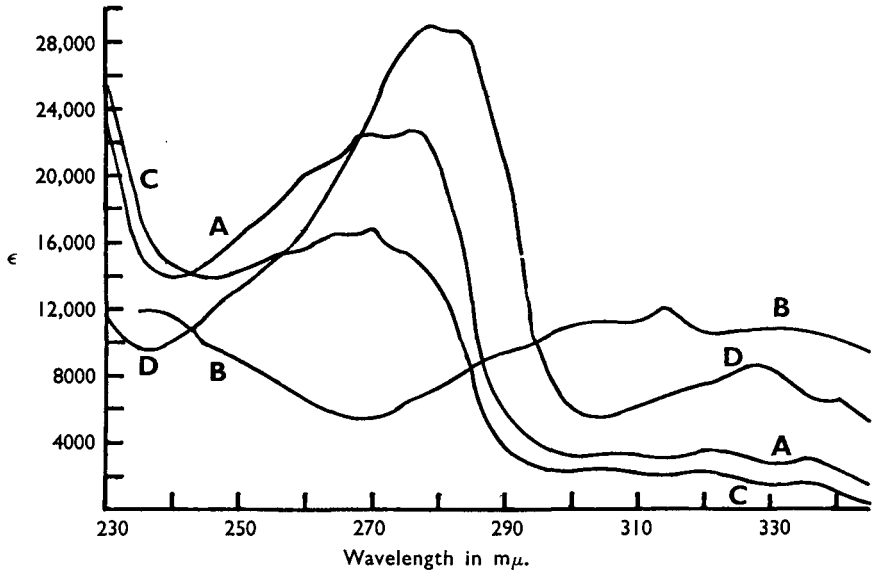


FIG. 1. Ultra-violet absorption curves for phenylindanedione.

A. Ethanol.
B. *cyclo*Hexane.
C. Ethanol + N hydrochloric acid.
D. 0·1 N aqueous sodium hydroxide.

speed of bromination) and even the addition of acid by no means completely suppresses enolisation. Only the *cyclo*hexane solution yields a completely different curve, presumably that of the diketone. The $E_{\text{max. 1 cm.}}^{\text{1 per cent.}}$ figure (1299) for the peak at 278 mμ in alkaline solution differs somewhat from that given in the literature (1328)[1].

TABLE III

RESULTS OF SPECTROPHOTOMETRIC MEASUREMENTS

Solvent	λ_{max} mμ	ϵ_{max}	$E_{max\ 1\ cm.}^{1\ per\ cent.}$
Ethanol (95 per cent.)	269	22,350	1006
" "	320	3423	154
0·1N sodium hydroxide (aqueous) ..	278	28,850	1299
" "	327	8570	386
N hydrochloric acid (ethanolic) ..	270	16,700	751
" " " ..	320	2218	99·8
*cyclo*Hexane	314	11,900	536

EXPERIMENTAL

Determination of phenylindanedione by oxidation

About 300 mg. of sample, accurately weighed, was dissolved in 20 ml. of N sodium hydroxide in a flask fitted with an air condenser (to minimise loss of spray) and 20 ml. of solution of hydrogen peroxide (100 volumes) added. The flask was swirled until the solution was colourless and then heated on a water bath to decompose the excess of peroxide. After

effervescence had ceased the flask was cooled to room temperature and the excess of alkali titrated against 0·5N hydrochloric acid, using phenolphthalein as indicator. The operation was repeated without the phenylindanedione. The difference between the two titrations represents the number of ml. of 0·5N alkali required to neutralise the acids produced by the oxidation of the phenylindanedione. Each ml. of 0·5N hydrochloric acid is equivalent to 0·03350 g. of $C_{15}H_{10}O_2$ (Table I). (This is an arbitrary factor; if one molecule of phenylindanedione yielded exactly one molecule each of benzoic and phthalic acids, then 1 ml. of 0·5N hydrochloric acid would be equivalent to 0·03703 g. of $C_{15}H_{10}O_2$.)

Examination of oxidation products

(*a*) *Qualitative.* The neutral solution obtained from the assay was evaporated to low bulk and acidified with hydrochloric acid. Some carbon dioxide was evolved and a white precipitate was produced. This gave positive phenolphthalein and fluorescein tests with concentrated sulphuric acid and phenol and resorcinol respectively, but no indication could be obtained of any other acids. The precipitated acid was shaken with ether (in which phthalic acid is sparingly soluble) and the ethereal solution was separated. The residual material, on drying, melted at 190° to 200° C. and on recrystallisation from ethanol yielded hard white needles which melted (sealed tube) at 203° C. Genuine phthalic acid under the same conditions melted at 206° C. Mixed m.pt. 205° C. Positive reactions were obtained from the recrystallised material with the usual tests for phthalic acid.

Evaporation of the ethereal solution yielded a white crystalline residue melting at 120° to 155° C. After two recrystallisations from hot water the product melted at 121° to 123° C. Mixed m.pt. with genuine benzoic acid, 123° C.

(*b*) *Quantitative.* 0·381 g. of phenylindanedione was oxidised in the usual way and then acidified and extracted completely with ether. The ethereal layer was washed with a little ferrous sulphate solution to remove peroxides, followed by a little water and the bulk of the ether removed on a warm water bath. The residue was dried to constant weight (several days) in a desiccator. Yield of mixed acids, 0·416 g. (84 per cent. of theory).

The whole of the dry acidic material was dissolved in neutralised ethanol and titrated with standardised 0·5N alkali using phenolphthalein. Found: 0·416 g. of mixed acids ≡ 8·45 ml. of 0·5N alkali. From this it was calculated that the mixture contained 49 per cent. of benzoic acid and 51 per cent. of phthalic acid or 0·204 g. of benzoic acid (97·5 per cent. of theory) and 0·214 g. of phthalic acid (75·3 per cent. of theory). The remaining 24·7 per cent. of the required amount of phthalic acid had been further oxidised by the peroxide, thus accounting for the high titre and the presence of carbon dioxide in the oxidation products.

Determination of phenylindanedione by bromination

About 300 mg. of sample was dissolved in 50 ml. of ethanol (95 per cent.) with the aid of heat, and the solution cooled to room temperature. 10 ml.

of a 10 per cent. v/v solution of bromine in ethanol (95 per cent.) was added and the whole left for 5 minutes with occasional swirling. 1 g. of β-naphthol was added and the flask shaken until the colour of the bromine had been discharged, any traces of bromine vapour being removed by blowing into the flask. 50 ml. of water and 10 ml. of solution of potassium iodide were added and the liberated iodine titrated with 0·1N sodium thiosulphate, using starch solution as indicator. At the end-point the solution has the orange colour of phenylindanedione. Each ml. of 0·1N sodium thiosulphate is equivalent to 0·01111 g. of $C_{15}H_{10}O_2$. (See Table II.)

Spectrophotometric examination of phenylindanedione

The determinations were carried out on a Hilger "Uvispek" spectrophotometer using 1 cm. cells and the hydrogen arc. Strengths of solutions examined were (*a*) 2·0 mg. per 100 ml. and (*b*) 0·5 mg. per 100 ml., in all 4 solvents. All solutions were freshly prepared without the use of heat.

Summary

1. Attempts to assay phenylindanedione by a gravimetric technique using dinitrophenylhydrazine met with failure to obtain consistent results.

2. Alkaline solutions of hydrogen peroxide oxidise the material to phthalic acid and benzoic acid, the former being partly destroyed by further oxidation. Consistent results are obtained if the weight of sample taken does not exceed about 450 mg. The partial destruction of the phthalic acid renders an arbitrary factor necessary.

3. Bromination, followed by debromination with potassium iodide and titration of the liberated iodine gives excellent results.

4. The ultra-violet absorption curves of phenylindanedione in various solvents have been obtained, but except where only minute quantities of sample are available or where foreign matter such as tablet excipient is present, there is little to recommend a spectrophotometric assay.

The author wishes to thank Dr. V. Askam of the School of Pharmacy, University of London, for suggesting quantitative bromination as a means of assay.

References

1. *J. Amer. Med. Ass.*, 1953, **152**, 142.
2. Sharp, *Analyst*, 1951, **76**, 219.
3. Meyer, *Annalen*, 1911, **380**, 212.