THE ASSAY OF STILBOESTROL BY THE ISOTOPE DILUTION TECHNIQUE

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THE isotope dilution technique has often been used for analysing complex mixtures. In principle a small amount of radioactive compound which is isotopic with and in the same chemical form as the compound to be determined is added to the mixture of organic compounds. A pure derivative, is then isolated not necessarily completely, and its radioactivity measured. From the change in radioactivity per unit weight, specific activity, it is possible to calculate the quantity of compound present in the original mixture.

To a sample of stilboestrol was added ^{14}C labelled stilboestrol of known specific activity and the mixture was acetylated according to the B.P. 1958. The specific activity of a portion of the acetylated derivative was then determined, and from the change in activity the purity of the stilboestrol was calculated.

The measurement of ^{14}C ($\beta = 0{\cdot}155$ MeV) presents difficulty because of the low energy of the β-particle emitted, and it is only recently that the liquid scintillation technique has been introduced as a method of counting low energy β-particles. In this technique the sample containing the radioactive substance is added to a solution of the phosphor and the light flashes emitted by this mixture of substances dissolved in an organic solvent, when irradiated, are detected and amplified by a photomultiplier and then counted.

Davidson and Feigelson[1], and Bell and Hayes[6], have reviewed the applications and instrumentation of liquid scintillation counting respectively, and Stitch[2] has determined the variables affecting the method with particular reference to ^{14}C steroids. One advantage over previous methods is that the fixed 4Π geometry makes the method highly sensitive and reproducible.

Description of the Apparatus

The phosphor consisted[3] of naphthalene 80 g., diphenyloxazole (P.P.O.) 5 g., 1,4-di-2-(5-phenyloxazolyl)benzene 0·05 g., xylene 390 ml., 1,4-dioxane 390 ml., ethanol 235 ml. The reagent grade naphthalene was recrystallised from 80 per cent ethanol. The xylene and dioxane were refluxed with, and distilled from sodium. The absolute ethanol was dried by a method of Vogel[4]. The mixed solute was dissolved in 700 ml. of solvent and made up to 1 litre with additional solvent and was stored in a dark brown bottle in the refrigerator.

The photomultiplier tube (E.M.I. 6097s) was selected because of its low dark current and housed in a lead castle designed for liquid scintillation counting (Panax SC/LP). The castle was maintained at 5° to reduce the dark current to a minimum. The radioactive sample was added to the phosphor which was contained in a counting jar. The jar was immersed in silicone oil (MS200/20) to ensure a good light path between the bottom of the jar and the top of the photomultiplier tube. No air bubbles were allowed beneath the jar as this impairs the efficiency of the counter. When the castle door was closed a shutter mechanism between the counting jar and the photomultiplier tube opened and allowed light flashes to reach the photomultiplier. The pulses obtained were amplified with a Panax 4250 low gain amplifier (× 1,000) and then counted with a Panax D657C scaler operated with a Panax T300 timer. The optimum working conditions were found to be 1,450 volts with the discriminator set at 12 volts. The resolution time of the first dekatron tube (GC10D) was 50 microseconds.

TABLE I

Reference sample counts sec^{-1} corrected for lost counts and background A	Weight of stilboestrol g. X	Weight of acetylated stilboestrol g. W	Count rate of acetylated sample counts sec^{-1} corrected for lost counts and background B	Stilboestrol per cent $= \frac{A}{B} \times \frac{W}{X} \times 100 \times \frac{268{\cdot}4}{352{\cdot}4}$
1. 6429*	1·4771	0·4550	1,486	101·5
2. 3144	1·5000	0·1162	187	99·2
3. 3253	1·7510	0·0989	141	99·2
4. 3419	1·6308	0·2100	344	97·5

* 0·060 ml. of active stilboestrol solution.

Assay details. The ^{14}C labelled stilboestrol (Radiochemical Centre, Amersham) was dissolved in ethanol and its specific activity was approximately 2μc per ml.

The background count per 100 seconds with the phosphor alone in the jar was first determined. To the phosphor was then added 0·030 ml. (= 5μg.) of active stilboestrol solution using an "Agla" syringe, and the count rate was redetermined giving the rate of the reference solution.

Approximately 1·5 g. of Stilboestrol B.P. (Ward Blenkinsop) and 0·030 ml. of active stilboestrol solution were acetylated according to the B.P. method[5]. The acetylated stilboestrol was filtered on a No. 3 sintered glass filter and then dried at 110° for 1 hour. Approximately 100 mg. of this precipitate, accurately weighed was added to a sample jar containing phosphor and the count rate per 100 seconds determined. The acetylated stilboestrol was very soluble in the phosphor and amounts up to 520 mg. added to 12 ml. of phosphor (capacity of the counting jar) did not quench the light pulse. The results are shown in Table I.

The preliminary results show that the method should be useful for the assay of steroids which do not acetylate quantitatively, provided that the acetylated derivative (or any other suitable derivative) can be obtained in a pure form, for example, by repeated recrystallisation. The method suffers from the same limitations as the B.P. assay of stilboestrol in that up to 5 per cent monoacetylmonomethyl stilboestrol will pass undetected.

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After the Author presented the paper there was a DISCUSSION.