CHROM. 4187

The use of iodine as a non-destructive location reagent for steroids in thin-layer chromatography

Iodine has been widely used as a location reagent for steroids in both paper[1,2] and thin-layer[2] chromatography (TLC). Although it is non-specific, it is of value as a "non-destructive" means of location for compounds that do not absorb UV light; for example, when a zone is to be located and removed for subsequent characterisation.

During an examination of estrone by TLC it was discovered that iodine vapour produced an irreversible reaction to give monoiodo and diiodo derivatives[3]. VARON *et al.*[4] found that after only 10 sec exposure to iodine vapour less than 50% of each of three estrogens, including estrone, could subsequently be removed. This was using 5 μg quantities on Brinkmann Silica Gel H.

It was therefore decided to investigate a wide range of steroids in an attempt to classify those types that were susceptible to permanent modification by iodine. It has been suggested[5] that iodine sensitive compounds are generally found to be Lewis bases.

There were two problems that emerged during the investigation. The first was the length of time that should be given, both in exposing the plate to iodine vapour and in allowing the iodine to evaporate off (termed the recovery time). Recommended exposure times vary from a few seconds[6] to 10 min[7], and for steroids a period of 5–10 min is often necessary to obtain optimum sensitivity. It was decided to use 30 min as the standard exposure time, and 17 h (*i.e.* overnight) as the standard recovery time. This length of exposure was excessive, but was considered necessary to ensure that any permanent derivative formed would be detected.

The detection of derivatives was the second problem, and although IR and UV spectroscopy were both used, TLC was found to be of most value. IR spectroscopy is not satisfactory when insoluble steroids such as the corticosteroids have to be examined as nujol mulls, since the mulls cannot be prepared quantitatively and spectra can vary with the physical state of the compound. The measurement of the UV extinction coefficient of a small amount of material is susceptible to the presence of impurities from the adsorbent. Such impurities might also be detected during TLC but should be distinguishable by running always in the same position for a given solvent system. This was not observed. There is evidence only in one case that the adsorbent, which was silica gel, itself caused degradation of the steroid examined.

Experimental

Steroids were dissolved in the minimum volume of chloroform–methanol (1:1). Where possible 25 or 50 mg of steroid were used, but in some instances only 5 or 10 mg were available. The solution was streaked across a 10 × 20 cm plate which was placed in a TLC tank saturated with iodine vapour (and enclosed in a polythene bag), and left for 30 min. The plate was then placed in a fume hood overnight to remove the iodine, and the area containing steroid was removed by scraping off the adsorbent with a spatula. The adsorbent was extracted three times by swirling with cold chloroform, and the filtered solution was evaporated using a water pump and finally a high vacuum pump, with warming on a steambath.

The approximate weight of recovered material was noted but no attempt was

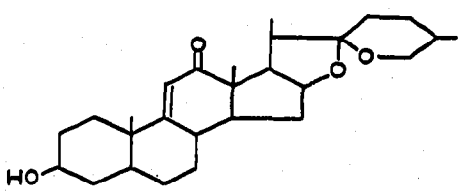
made to work quantitatively since there was no necessity to do so. The purpose of recording recoveries was to establish that a representative proportion of steroid was re-examined by TLC.

Thin-layer chromatography was done on a 0.25 mm thick layer of Silica Gel G (Macherey-Nagel), with the steroid dissolved as a 1% solution in chloroform–methanol (1:1). 2 λ spots were applied to the plate using a micropipette (Drummond microcap). Plates were run for 30 min, dried with a hairdryer, and sprayed with 25% ethanolic sulphuric acid. After heating for 10 min at 120° the plates were examined under UV light at 366 mμ. (In some cases iodine vapour itself was also used as the final location reagent.)

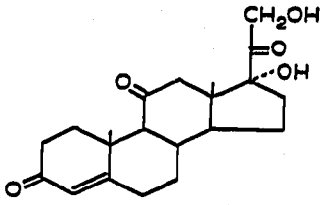
The following solvent systems were used: chloroform–ethyl acetate (1:1) (C_1EA_1); chloroform–ethyl acetate (4:1) (C_4EA_1); chloroform–ethyl acetate (9:1) (C_9EA_1); toluene–chloroform (1:1) (T_1C_1); benzene–ethyl acetate (4:1) (B_4EA_1).

Results and discussion

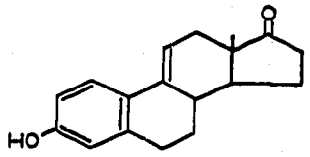
The results are collected in Table I. Steroids that did not appear to react with iodine vapour include sapogenins, both natural (hecogenin, diosgenin) and synthetic (Δ^9-hecogenin) (I), and corticosteroids and an androgen containing the 1,4-diene-3-ketone system.



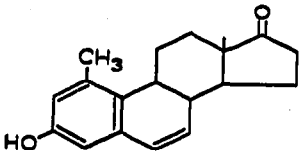
I



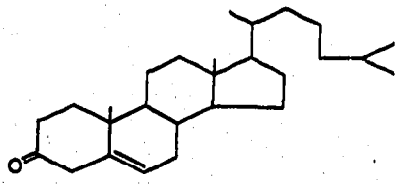
II



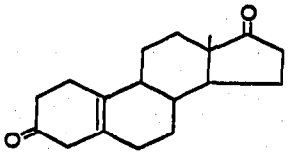
III



IV



V



VI

J. Chromatog., 43 (1969) 282–286

TABLE I

Steroid	*Wt. (mg)*		*Time (h)*		*TLC examination*	*Solvent system*[a]
	Applied	*Removed*	*Iodination*	*Recovery*		
Sapogenins						
Hecogenin	50	38	0.5	17	Mainly hecogenin; traces non-polar impurities, a little polar impurity	C_4EA_1
Δ^9-Hecogenin	50	37.4	0.5	17	Mainly Δ^9-hecogenin; small amounts polar and non-polar impurities	C_4EA_1
	40	37.6	5.0	17		
Diosgenin	50	50	0.5	17	Mainly diosgenin; minor non-polar impurities	C_9EA_1
	25	20.5	6.5	17		
Corticosteroids						
Cortisone	50	31	0.5	17	Mainly cortisone; traces polar impurities, slight non-polar smearing	C_1EA_1
	50	50	5.0	17		
Prednisone	25	18.5	0.5	17	Mainly prednisone; minor non-polar impurities	C_1EA_1
Prednisone acetate	50	38	0.5	17	No evidence of impurities	C_1EA_1
Androgens						
Androsta-1,4-diene-3,17-dione	25	22	0.5	17	No evidence of impurities	B_4EA_1
	50	47	5.0	48		
Dehydroepiandrosterone	50	39.7	0.5	17	Mainly DHEA, little non-polar	C_4EA_1

Cholesterol	50	39.4	0.5	17	No evidence of impurities	T_1C_1
Cholest-5-en-3-one	25	24	17	48	Mainly polar products	T_1C_1
Estrogens						
Estrone[3]	10	10	0.5	17	Mainly estrone; about 10% non-polar impurity	C_9EA_1
$\Delta^{9(11)}$-Estrone	0.8	(0.8)	0.5	17	Mainly $\Delta^{9(11)}$-estrone; 5% non-polar mixture, 10% polar impurity	C_4EA_1
Estrone methyl ether	20	19.3	0.5	17	Mainly estrone methyl ether; about 10% non-polar impurity	C_1T_1
1-Methyl-Δ^6-estrone	10	10	5.0	17	Main product less polar, several minor impurities	C_1EA_1
Estr-5(10)ene-3,17-dione	5	5	0.5	17	About 10% polar and 5% non-polar impurity	B_4EA_1
	25	25	5.0	17	Main product more polar; <5% estrene-dione	C_1EA_1

[a] See *Experimental*.

MATTHEWS *et al.*[6] noted that cortisone (II) gave a new compound after a few seconds exposure to iodine. Our investigation showed that even after 5 h exposure, only traces of more mobile impurities were observed on TLC, and IR evidence confirmed that the cortisone was largely unaffected. This appears to be true also for prednisone, both as the free alcohol and as the 21-acetate.

The reactive steroids appear in the lower half of Table I and include a variety of estrogens. By analogy with the materials isolated from estrone[3], these products are assumed to arise by iodination of the aromatic ring A nucleus. The two phenolic styrenes Δ^9-estrone (III) and 1-methyl-Δ^6-estrone (IV) showed a number of polar transformation products as well. β,γ-Unsaturated ketones were investigated, and almost complete transformation of this system was observed after location with iodine vapour. Thus cholest-5-en-3-one (V) isomerised to the conjugated 4-en-3-one, and the UV spectrum of the isolated material suggested that some 4,6-dien-3-one was also formed (λ_{max} 240 and 284 mμ). It has been found, however, that cholest-5-en-3-one will isomerise on silica gel alone in the absence of iodine vapour, although under these conditions no 4,6-dien-3-one is observed. The rate of isomerisation is increased by exposing the silica gel plate to iodine. Another β,γ-unsaturated ketone, estr-5(10)-ene-3,17-dione (VI) was rapidly converted into a mixture of less mobile and slightly more mobile products, corresponding in mobility to estra-4,9-diene-3,17-dione and estrone, respectively. With this 5(10)-3-ketone there was negligible isomerisation on silica gel alone.

It is clear, then, that significant chemical transformations can occur in the iodine tank, and that in a number of cases iodine does not simply form loose complexes with compounds adsorbed on TLC plates. This may also be the case in several of the steroids shown here to be recovered unchanged after exposure to iodine. It may be particularly true of compounds containing unconjugated double bonds, such as 5-en-3-ols, in which the survival of the double bond could be due to its regeneration following ready reversibility of addition of the halogen.

Technical assistance in this work was given by Mr. J. B. ADAMS (Glaxo Laboratories Ltd.) and is gratefully acknowledged.

Glaxo Laboratories Ltd., Montrose, Angus (Great Britain) PETER J. STEVENS

Department of Chemistry, University of Aberdeen, Aberdeen (Great Britain) ALAN B. TURNER

1 I. E. BUSH, *Chromatography of Steroids*, Pergamon, London, 1961.
2 R. NEHER, *Steroid Chromatography*, Elsevier, Amsterdam, 1964.
3 W. BROWN AND A. B. TURNER, *J. Chromatog.*, 26 (1967) 518.
4 H. H. VARON, H. A. DARNOLD, M. MURPHY AND J. FORSYTHE, *Steroids*, 9 (1967) 507.
5 S. DITTRICH, *J. Chromatog.*, 31 (1967) 628.
6 J. S. MATTHEWS, A. L. PEREDA V. AND A. AGUILERA P., *J. Chromatog.*, 9 (1962) 331.
7 B. V. MILBORROW, *J. Chromatog.*, 19 (1965) 194.

Received March 17th, 1969