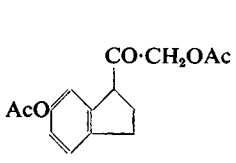
SYNTHETIC ANALOGUES OF ADRENAL CORTICAL HORMONES—DERIVATIVES OF FLUORENE AND INDANE

BY V. ASKAM, W. H. LINNELL, and J. VORA;
with a note by P. F. D'ARCY.

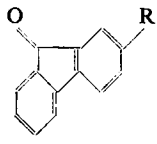
From the Chemistry Department, School of Pharmacy, 17 Bloomsbury Square, London, W.C.1

Received January 6, 1956

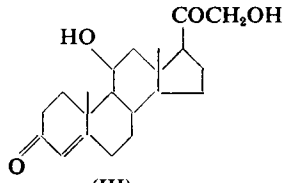
NUMEROUS synthetic analogues of adrenal cortical hormones have been reported in the literature, but few possess an oxygen atom at the equivalent of the 11-position of the steroid nucleus i.e. separated from the characteristic side chain $-COCH_2OH$ by four carbon-carbon bond lengths[1,2]. In these laboratories, aromatic and hydroaromatic analogues containing such an oxygen atom have been prepared. The present paper reports the preparation of 1-acetoxyacetyl-6-acetoxyindane (I), 2-acetoxyacetylfluoren-9-one (II) and 2-hydroxyacetylfluoren-9-one (IIa). The structural similarities of these compounds to corticosterone (III) are shown in the formulæ.



(I)



(II)

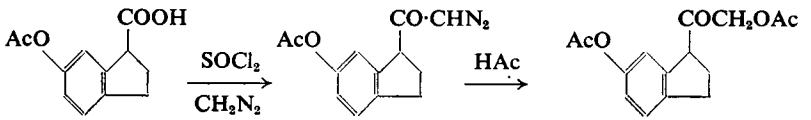


(III)

(II, R = $COCH_2OAc$; IIa, R = $COCH_2OH$; IIb, R = $COCH_3$; IIc, R = $COCH_2Br$)

The compounds were biologically tested in mice for adrenal cortical hormone activity by the cold-stress and glycogen deposition tests. Interest was centred on the latter test in view of the relationship of the compounds to the 11-oxygenated adrenal hormones which have a prepotent effect on carbohydrate metabolism. Alternatively any synthetic analogue may, by combination with vital receptors, have the biological action of antagonism to the natural hormone. In an attempt to obtain indications of any such antagonism, cold-stress tests were made in which the compounds (I) and (IIa) were administered together with cortisone acetate. An interesting example of this kind appears in the reported antagonism of a compound to the glyconeogenetic action of cortisone acetate[3]. The results of the biological tests are appended.

1-Acetoxyacetyl-6-acetoxyindane was prepared from 6-acetoxyindane-1-carboxylic acid (IV)[4], via the diazoketone as follows:—



(IV)

The diazoketone procedure could not be used for the preparation of the analogue based on the fluorenone structure; ring expansion to derivatives of phenanthrene[5] occurs when fluorenone is treated with diazomethane. The following method was therefore adopted.

Oxidation of 2-acetylfluorene using sodium dichromate and acetic acid by the method of Ray and Rieveschl[6] gave fluoren-9-one-2-carboxylic acid together with a small amount of alkali-insoluble material which these workers had not identified. This material was evidently a product of partial oxidation of 2-acetylfluorene since when milder oxidation conditions were used, its yield was increased to 60 per cent. Moreover, on further oxidation, the compound gave fluorenone-2-carboxylic acid in good yield.

The constitution of the compound was established as 2-acetylfluoren-9-one (IIb) by the fact that it was identical with a specimen prepared from the acid chloride of fluorenone-2-carboxylic acid by the action of sodio-malonic ester followed by hydrolysis and decarboxylation.

Bromination of 2-acetylfluoren-9-one (IIb) gave the bromoacetyl compound (IIc) which, on treatment with potassium acetate was converted into the required α-ketol acetate (II). This was hydrolysed by the method of Mattox and Kendall[7] to give the free α-ketol (IIa). When oxidised, compounds (IIc) and (IIa) both gave fluorenone-2-carboxylic acid, thus showing that substitution by bromine had occurred, as expected, in the side chain and not in the fluorenone nucleus.

Examination of compounds (I), (II) and (IIa) for adrenal cortical activity. These tests were made by P. F. D'Arcy. (By courtesy of Prof. G. A. H. Buttle.)

Compounds (I) and (II) were tested for adrenal cortical activity by a mouse cold-stress method. Details of this test have been published elsewhere[8]. These compounds, when injected intramuscularly into groups of 10 adrenalectomized mice, failed to afford a significant protection against the effects of cold stress although they were administered over a wide range of doses extending to a toxic level.

Compounds (I) and (IIa) were administered intramuscularly in aqueous suspension together with cortisone acetate in doses of respectively 0·25 mg. and 1·0 mg. per mouse. The compounds showed slight activity in potentiating the activity of a range of doses of cortisone acetate: statistical examination however showed that this potentiation was not significant.

From the above results, it was concluded that under the conditions of the test, compounds (I), (II) and (IIa) had no significant adrenal cortical activity and compounds (I) and (IIa) no antagonism to cortisone acetate.

Compounds (I) and (II) were examined for adrenal cortical activity by the mouse liver glycogen deposition test of Venning, Kazmin and Bell[9]. Both compounds, when injected subcutaneously in aqueous suspension into groups of 9-10 adrenalectomized animals in doses of 0·25 mg. and 1·0 mg. failed to cause a significant deposition of liver glycogen.

EXPERIMENTAL

All m.pts. are uncorrected.

1-Acetoxyacetyl-6-acetoxyindane. 6-Acetoxyindane-1-carboxylic acid[4] (10 g.) was refluxed with pure thionyl chloride (20 ml.) and dry benzene (50 ml.). When hydrogen chloride was no longer evolved, the benzene and thionyl chloride were removed by heating in an oil bath under reduced pressure. The last traces of thionyl chloride were removed by adding dry benzene and again removing under reduced pressure. The residue was distilled at 0·5 mm. pressure with air bath heating in a distillation apparatus with low side arm, the flask being packed with glass wool. The acid chloride distilled as a pale yellow liquid. (7·5 g.). This gave a *p*-toluidine which crystallised as cream coloured needles from aqueous ethanol, m.pt. 196–197° C. Found: C, 73·5; H, 6·2; N, 4·6. $C_{19}H_{19}O_3N$ requires C, 73·7; H, 6·19; N, 4·53 per cent.

The acid chloride (7 g.) was dissolved in a little dry ether and the solution gradually added to an ethereal solution of diazomethane prepared by the standard method[10], from nitrosomethylurea (20 g.). The solution was set aside for three hours then the ether and diazomethane removed by distillation under reduced pressure at room temperature. The diazoketone crystallised as a yellow solid when the greater part of the liquid had been removed, and was separated by filtration. After washing with dry ether, 4·8 g. of crude diazoketone remained.

The crude diazoketone (1·5 g) was heated on a steam bath with glacial acetic acid (7·5 ml.) until nitrogen was no longer evolved. The mixture was poured into water and extracted with ether. The ethereal solution was washed well with water, then with cold 1 per cent. sodium bicarbonate solution, then again with water. The volume of the ethereal solution was adjusted to 100 ml., and after drying with anhydrous sodium sulphate the solution was passed through a column of activated charcoal (10 cm. × 1·5 cm.). A further 100 ml. of ether was then passed through the column; evaporation of the ether gave a colourless product (1·1 g.). This was crystallised from a mixture of benzene and petroleum ether (40–60°C.) to give *1-acetoxyacetyl-6-acetoxyindane* as colourless prisms m.pt. 66·5–67·5°C. Found: C, 65·3; H, 5·9. $C_{15}H_{16}O_5$ requires C, 65·2; H, 5·84 per cent. The compound readily reduced warm Fehling's and Tollen's reagents.

2-Acetylfluorene was prepared by the method of Ray and Rieveschl[11]. The crude substance was found to be sufficiently pure for oxidation at the next stage.

Fluoren-9-one-2-carboxylic acid. Crude 2-acetylfluorene (45 g.) when oxidised by the method of Ray and Rieveschl[6] gave fluorene-9-one-2-carboxylic acid (26 g.) m.pt. 338–340° C. (decomp.) together with an alkali insoluble material (4 g.).

2-Acetylfluoren-9-one. (i) Fluoren-9-one-2-carboxylic acid (20 g.) was refluxed on a water bath for three hours with pure thionyl chloride (70 ml.) and dry benzene (250 ml.). The benzene and excess of thionyl chloride were removed by heating under reduced pressure, and the residue dissolved in dry benzene (250 ml.). The solution was gradually

added to a cooled suspension of sodio-malonic ester prepared by refluxing for 7 hours a mixture of powdered sodium (4·2 g.), diethyl malonate (43 g.), and dry ether (500 ml.). The mixture was stirred for 6 hours at room temperature, refluxed for 3 hours, cooled, diluted with water and then acidified with acetic acid. The ethereal layer was separated and ether removed by distillation. In order to decarboxylate the residue, it was heated under reflux for 4 hours with hydrochloric acid (250 ml.), water (100 ml.) and acetic acid (250 ml.). Water (1500 ml.) was then added and the crude 2-acetylfluoren-9-one removed by filtration. The filtrate was extracted with benzene and the solid dissolved in the benzene solution. This was washed with 5 per cent. potassium bicarbonate solution and then with water. After removal of the benzene, the residue was crystallised from ethanol-benzene mixture to give *2-acetylfluoren-9-one* (14·5 g.) as yellow needles m.pt. 162–163°C. Found: C, 81·6; H, 4·7; $C_{15}H_{10}O_2$ requires C, 81·1; H, 4·5 per cent.

The 2:4-*dinitrophenylhydrazone* prepared by the usual method was evidently a mixture of mono and bis derivatives. Several crystallisations from nitrobenzene-acetic acid mixture gave dark red needles m.pt. 300°C. (decomp.). Found: C, 63·2; H, 4·1; N, 14·2. $C_{21}H_{14}O_5N_4$ requires C, 62·7; H, 3·5; N, 13·9 per cent.

(ii) Sodium dichromate (250 g. coarsely powdered) was added in 5 g. portions over a period of 45 minutes to a stirred solution of 2-acetylfluorene (50 g.) in glacial acetic acid (700 ml.). Throughout the addition, the mixture was maintained at a constant temperature by a water bath at 50–55° C. The mixture was stirred at this temperature for a further 10 hours, then poured into hot water (3 litres).

The suspension was well shaken in a mechanical shaker, cooled, filtered and the residue washed with dilute sulphuric acid (2 per cent.). The product was stirred with 5 per cent. potassium hydroxide solution (700 ml.) and the suspension filtered. The residue was washed with water, dried and crystallised twice from ethanol then once from benzene to give *2-acetylfluoren-9-one* (34 g., 60 per cent.) m.pt. 162–163°C. undepressed on admixture with sample from route (i) Found: C, 81·2; H, 4·7. $C_{15}H_{10}O_2$ requires C, 81·1; H, 4·5 per cent.

2-Bromoacetylfluoren-9-one. 2-Acetylfluoren-9-one (11·1 g.) was dissolved in chloroform (100 ml.) in a ½ litre three necked flask fitted with a dropping funnel condenser and mercury-seal stirrer, and illuminated by two 100-watt electric lamps. The mixture was heated to gentle reflux by means of a water bath, and two or three drops of a solution of hydrobromic acid in acetic acid (50 per cent.) added. The mixture was stirred and a solution of bromine in chloroform (0·5 molar 100 ml.) added drop by drop during 2 hours. After a further 3 hours, heating was discontinued but stirring and illumination maintained for a further 12 hours. The chloroform was then removed under reduced pressure and the residue twice crystallised from benzene to give *2-bromoacetylfluorenone* as pale yellow needles (13 g.) m.pt. 208–209°C. (decomp.) Found: C, 59·2; H, 3·0; Br, 26·9. $C_{15}H_9O_2Br$ requires C, 59·8; H, 3·0; Br 26·5 per cent.

2-Acetoxyacetylfluoren-9-one. The above bromoacetyl compound (5 g.) was dissolved in dry acetone (250 ml.) and freshly fused and powdered potassium acetate (10 g.) added. The mixture was gently refluxed for 6 hours and then set aside for two days and filtered, the residue being washed with a little dry acetone. The filtrate and washings were treated with activated charcoal, filtered and the acetone removed under reduced pressure. The residue (3·75 g.) was crystallised from dry benzene to give *2-acetoxyacetylfluoren-9-one* as yellow needles, m.pt. 164–165°C. Found: C, 73·2; H, 4·3. $C_{17}H_{12}O_4$ requires C, 73·0; H, 4·3 per cent. The product reduced warm Fehling's and Tollen's reagents.

2-Hydroxyacetylfluoren-9-one. 2-acetoxyacetylfluoren-9-one (0·7) g.) was dissolved in a mixture of chloroform (15 ml.) and methanol (25 ml.). Water, (1 ml.) was added, the solution cooled to 10° C., and hydrochloric acid, (2 ml.) slowly added so that the temperature did not rise above 20°C. Golden yellow needles immediately separated from the solution which was set aside at room temperature for 24 hours. A further portion (1 ml.) of hydrochloric acid was added. When crystals were no longer deposited, the mixture was filtered, and the product dried *in vacuo*. Crystallisation from benzene gave *2-hydroxyacetylfluoren-9-one* as yellow needles (0·55 g) m.pt. 174–175°C. Found: C, 75·2; H, 4·2; $C_{15}H_{10}O_3$ requires C, 75·6; H, 4·2 per cent. The compound readily reduced warm Fehling's and Tollen's reagents.

Oxidation experiments. 2-Bromoacetylfluoren-9-one and 2-acetoxyacetylfluoren-9-one were oxidised following procedures similar to that used in the oxidation of 2-acetylfluorene. The compounds gave fluoren-9-one-2-carboxylic acid m.pt. 340° C. in yields greater than 70 per cent.

SUMMARY

1. 1-Acetoxyacetyl-6-acetoxyindane (I), 2-acetoxyacetylfluoren-9-one (II), and 2-hydroxyacetylfluoren-9-one (IIa) have been prepared and tested for adrenal cortical activity.

2. Compounds (I) and (II) did not exhibit significant activity when tested by the mouse cold-stress or liver glycogen tests.

3. Compounds (I) and (IIa) showed slight activity in potentiating the action of cortisone acetate in the cold-stress test: this activity was not, however, significant.

REFERENCES

1. Ross, *J. chem. Soc.*, 1945, 538.
2. Walker, *J. chem. Soc.*, 1942, 347.
3. Adams, Cross, David, Hartley, Patel, Petrow and Stuart-Webb, *J. Pharm. Pharmacol.*, 1953, **5**, 861.
4. Askam and Linnell, *J. chem. Soc.*, 1954, 4691.
5. Schultz, Schultz and Cochran, *J. Amer. chem. Soc.*, 1940, **62**, 2902.
6. Ray and Rieveschl, *Organic Syntheses*, **28**, 63.
7. Mattox and Kendall, *J. biol. Chem.*, 1951, **188**, 293.
8. Buttle, D'Arcy and Howard, *J. Physiol.*, 1954, **123**, 5P.
9. Venning, Kazmin and Bell, *Endocrinol.*, 1946, **38**, 79.
10. Adams, "*Organic Reactions*" (Wiley 1942) **1**, 50.
11. Ray and Rieveschl., *Organic Syntheses*, **28**, 3.