SUMMARY

1. A series of fifteen 3:5-diiodo-4-alkoxybenzoic acids and their esters has been tested for antithyroxine activity by the oxygen consumption method in mice, but all were markedly inferior to *n*-butyl 3:5-diiodo-4-hydroxybenzoate.

2. Four branched-chain alkyl esters of 3:5-diiodo-4-hydroxybenzoic acid were found to be much less active than the straight-chain esters.

3. 3:5-Diiodo-4-methoxybenzonitrile exerted slight antithyroxine activity, but 3:5-diiodo-4-*n*-propoxybenzonitrile and the corresponding benzamide were inactive.

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The Biological Action of Substances Related to Thyroxine

3. SUBSTANCES DERIVED FROM 3:5-DIODO-4-HYDROXYBENZALDEHYDE AND RELATED COMPOUNDS

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The dimethylacetal of 3:5-diiodoanisaldehyde (Wilkinson, 1949) was shown by MacLagan, Sheahan & Wilkinson (1949) to exhibit an antithyroxine action when tested in mice by the oxygen-consumption method (MacLagan & Sheahan, 1950). This effect was only produced at a total dosage of 1000 mg./kg. and it was the object of the work described in the present communication to survey a series of related compounds in the hope of finding more effective substances. Frieden & Winzler (1949) have shown that 3:5-diiodo-4-benzyloxybenzoic acid was active by the amphibian-metamorphosis test, a result we have confirmed in mice by the oxygen-consumption test (Wilkinson, Sheahan & MacLagan, 1951). Accordingly, we decided to examine the benzyl ether of 3:5-diiodo-4-hydroxybenzaldehyde and its dimethylacetal, but as frequently occurs when two supposedly active groups are combined in one compound, the products had no pharmacological action. We therefore turned our attention to a more systematic study of derivatives of a series of ethers of 3:5-diiodo-4-hydroxybenzaldehyde and related substances.

The ethyl and benzyl ethers of the hydroxy aldehyde were obtained by the methods used for the preparation of the corresponding ethers of the diiodohydroxybenzoates (Wilkinson *et al.* 1951). The ethyl ether was characterized as its oxime and

dimethyl acetal, and the benzyl derivative was converted into its dimethyl acetal by the usual procedure. By similar means 3:5-diiodo-2-methoxybenzaldehyde and its dimethyl acetal were prepared from 3:5-diiodosalicylaldehyde. Some esters of 3:5-diiodo-4-hydroxybenzaldehyde were obtained by standard methods and some of these were readily converted into the corresponding acetals.

In order to determine whether mono-iodo substituted compounds were likely to be of any interest we prepared 5-iodovanillin and 5-iodoveratraldehyde. The latter was obtained by the methylation of the former by the method of Kalb, Schweizer, Zellner & Barthold (1926) using a large excess of methyl sulphate. Less drastic conditions failed to produce more than a trace of the required veratraldehyde.

EXPERIMENTAL

Chemical methods

Micro-analyses were by Drs Weiler and Strauss, Oxford. All melting points are uncorrected.

The following new derivatives of 3:5-diiodoanisaldehyde are reported:

Oxime. Colourless needles, m.p. 153°, from benzene. Highly soluble in most organic solvents, except light petroleum. (Found: C, 24.0; H, 1.7; I, 62.8. $C_9H_7O_2NI_2$ requires C, 23.8; H, 1.8; I, 63.1%.)

Diethyl acetal. Diiodoanisaldehyde (1 g.) was heated under reflux for 1 hr. with ethanol containing 1% (w/w) HCl (10 ml.) and, after cooling, the solution was treated with ice and Na_2CO_3 . The solid (0.89 g.) was collected and dried over calcium chloride. The compound crystallized from light petroleum (b.p. 40–60°) in colourless rhombic prisms, m.p. 76°. (Found: C, 31.2; H, 3.5; I, 55.4. $\text{C}_{12}\text{H}_{16}\text{O}_3\text{I}_2$ requires C, 31.2; H, 3.5; I, 55.0%.)

3:5-Diiodo-2-methoxybenzaldehyde. 3:5-Diiodosalicylaldehyde (10 g.) was suspended in methanol (70 ml.) and treated with dimethyl sulphate (16 g.). 40% NaOH (16 ml.) was added and, after the vigorous reaction had subsided, the mixture was set aside overnight. Water (200 ml.) was added and the acetal which separated was extracted with ether, dried (Na_2SO_4) and freed from solvent. As it could not be induced to crystallize and decomposed when distillation at 0.1 mm. was attempted, the acetal was hydrolysed by boiling with 2N-HCl (10 ml.) for 15 min. The aldehyde (5.42 g.), which solidified on cooling, separated from light petroleum (b.p. 80–100°) in colourless needles, m.p. 100°. (Found: C, 24.8; H, 1.7; I, 65.4. $\text{C}_8\text{H}_6\text{O}_3\text{I}_2$ requires C, 24.7; H, 1.6; I, 65.5%.) The dimethyl acetal was obtained from the purified aldehyde (1 g.) by refluxing with methanol containing 1% (w/w) HCl (10 ml.) for 1 hr., after which the solution was treated with ice and Na_2CO_3 . Extraction with ether furnished a yellow viscous oil (1.15 g.) which again failed to crystallize. (Found: C, 28.2; H, 2.9; I, 58.9. $\text{C}_{10}\text{H}_{14}\text{O}_3\text{I}_2$ requires C, 27.7; H, 2.8; I, 58.6%.)

3:5-Diiodo-4-ethoxybenzaldehyde, its oxime and dimethyl acetal were prepared as described by Wilkinson (1951).

3:5-Diiodo-4-benzoyloxybenzaldehyde. 3:5-Diiodo-4-hydroxybenzaldehyde (7.5 g., 0.02 g.mol.) was suspended in ethanol (50 ml.) and treated with 2N-NaOH (30 ml., 0.06 g.mol.). Benzyl chloride (7.6 g., 0.06 mol.) was added and the mixture boiled under reflux for 1 hr., when it was no longer alkaline to litmus. An oil, which hardened to a gum, separated on cooling. Repeated crystallization from 90% ethanol gave the required aldehyde (4.0 g.) as colourless needles, m.p. 103°. (Found: C, 36.2; H, 2.2; I, 55.2. $\text{C}_{14}\text{H}_{10}\text{O}_3\text{I}_2$ requires C, 36.2; H, 2.2; I, 54.7%.) The 2:4-dinitrophenylhydrazone separated from dioxan in orange-red needles, m.p. 263–265° (decomp.). (Found: N, 8.8. $\text{C}_{20}\text{H}_{14}\text{O}_5\text{N}_2\text{I}_2$ requires N, 8.7%.) The dimethyl acetal was obtained by heating under reflux a mixture of the aldehyde (2 g.) and anhydrous CaCl_2 (0.3 g.) in methanol (6 ml.) for 2 hr. The oil, which separated on dilution with water, hardened overnight, and, after crystallization from methanol, formed colourless plates, m.p. 95°. (Found: C, 37.6; H, 3.1; I, 49.9. $\text{C}_{16}\text{H}_{16}\text{O}_3\text{I}_2$ requires C, 37.7; H, 3.1; I, 49.8%.)

3:5-Dibromoanisaldehyde dimethyl acetal was obtained by treating a solution of 3:5-dibromo-4-hydroxybenzaldehyde (28 g.) in methanol (150 ml.) and dimethyl sulphate (50 g.) with 40% NaOH (50 ml.) as described under 3:5-diiodo-2-methoxybenzaldehyde. On dilution with water the acetal (23.15 g.) separated as an oil which, on treatment with ether, hardened to yellow solid, m.p. 58–59°. Recrystallization from light petroleum (b.p. 40–60°) gave pale-yellow plates, m.p. 62–63°. (Found: C, 35.8; H, 3.4; Br, 48.6. $\text{C}_{10}\text{H}_{12}\text{O}_3\text{Br}_2$ requires C, 35.3; H, 3.5; Br, 47.1%.) Hydrolysis with 2N-HCl by boiling under reflux for 20 min. gave the aldehyde in 97% yield. It crystallized from ethanol in colourless needles, m.p. 85°. (Found: C, 33.4; H, 2.2; Br, 54.1. $\text{C}_8\text{H}_6\text{O}_3\text{Br}_2$ requires C, 32.6; H, 2.1; Br, 54.4%.)

3:5-Diiodo-4-acyloxybenzaldehydes. 3:5-Diiodo-4-hydroxybenzaldehyde (9.35 g., 0.025 mol.) was dissolved in pyridine (15 ml.) and treated with the appropriate acid anhydride or acid chloride (0.03 mol.). After 30 min. at room temperature, the solution was heated to 60° for 10 min., cooled and diluted with water (50 ml.). The esters were usually obtained as gums, which hardened on standing, though in some cases treatment with ethanol was necessary to induce crystallization. (Yields: 80–95%.) By this means the following were prepared:

Acetate. Colourless needles, m.p. 158°, from 90% ethanol. (Found: C, 26.4; H, 1.5; I, 61.2. $\text{C}_8\text{H}_6\text{O}_5\text{I}_2$ requires C, 26.0; H, 1.45; I, 61.1%.)

The dimethyl acetal formed colourless prisms, m.p. 102°, from methanol. (Found: C, 28.6; H, 2.6; I, 55.0. $\text{C}_{11}\text{H}_{14}\text{O}_4\text{I}_2$ requires C, 28.6; H, 2.6; I, 55.0%.)

Propionate. Pale-buff crystals, m.p. 127°, from light petroleum (b.p. 80–100°). (Found: C, 28.2; H, 1.8; I, 58.8. $\text{C}_{10}\text{H}_8\text{O}_5\text{I}_2$ requires C, 27.9; H, 1.9; I, 59.1%.)

The dimethyl acetal separated from light petroleum (b.p. 40–60°) in colourless prisms, m.p. 54–55°. (Found: C, 29.8; H, 2.9; I, 53.6. $\text{C}_{12}\text{H}_{14}\text{O}_4\text{I}_2$ requires C, 30.3; H, 2.95; I, 53.4%.)

n-Butyrate. Pale-buff crystals, m.p. 81–82°, from light petroleum (b.p. 60–80°). (Found: C, 29.9; H, 2.1; I, 57.0. $\text{C}_{11}\text{H}_{10}\text{O}_5\text{I}_2$ requires C, 29.7; H, 2.3; I, 57.2%.)

Benzoate. Buff needles from ethanol, m.p. 138–140°. (Found: C, 35.1; H, 1.8; I, 52.8. $\text{C}_{14}\text{H}_8\text{O}_5\text{I}_2$ requires C, 35.2; H, 1.7; I, 53.1%.)

Phenylacetate. Buff crystals from ethanol, m.p. 133–134°. (Found: C, 36.8; H, 2.1; I, 51.5. $\text{C}_{15}\text{H}_{10}\text{O}_5\text{I}_2$ requires C, 36.6; H, 2.0; I, 51.6%.)

Toluene-p-sulphonate. Colourless prisms from 70% ethanol, m.p. 133°. (Found: C, 31.9; H, 2.1; I, 47.8. $\text{C}_{14}\text{H}_{10}\text{O}_4\text{I}_2\text{S}$ requires C, 31.8; H, 1.9; I, 48.1%.) The dimethyl acetal separated from benzene in colourless crystals, m.p. 198–199°. (Found: C, 34.7; H, 3.0; I, 43.8; S, 5.8. $\text{C}_{16}\text{H}_{16}\text{O}_5\text{I}_2\text{S}$ requires C, 33.5; H, 2.8; I, 44.2; S, 5.6%.)

3:5-Diiodo-4-hydroxypropiophenone. 4-Hydroxypropiophenone (1 g.) was suspended in NH_3 solution (sp.gr. 0.88; 10 ml.) and treated over 1.5 hr. with a solution of I_2 (3.4 g.) and KI (4 g.) in water (20 ml.). The mixture was set aside overnight, acidified with HCl, and filtered. The solid (2.54 g., 94%) crystallized from ethanol in colourless needles, m.p. 122°. (Found: C, 26.9; H, 1.8; I, 63.4. $\text{C}_9\text{H}_6\text{O}_4\text{I}_2$ requires C, 26.9; H, 2.0; I, 63.2%.) The acetyl derivative separated from ethanol in colourless highly refractive needles, m.p. 155°. (Found: I, 56.7. $\text{C}_{11}\text{H}_{10}\text{O}_5\text{I}_2$ requires I, 57.2%.)

3:5-Diiodo-4-methoxypropiophenone. The 4-hydroxy compound (4 g.) was suspended in a mixture of methanol (20 ml.) and methyl sulphate (3.8 g.) and treated with 10N-NaOH (3 ml.). The product (3.36 g., 81%) was isolated on dilution with water (50 ml.). It crystallized from light petroleum (b.p. 80–100°) in colourless rhombic plates, m.p. 83–84°. (Found: C, 28.6; H, 2.6; I, 61.1. $\text{C}_{10}\text{H}_{10}\text{O}_4\text{I}_2$ requires C, 28.8; H, 2.4; I, 61.1%.)

5-Iodovaleraldehyde. Iodovanillin (13.9 g.) was dissolved in methyl sulphate (50 ml.) at 100°. 20% NaOH was added gradually with vigorous shaking until the solution was alkaline to phenolphthalein. About 45 min. was required for the addition. The gum which separated crystallized on cooling. It was collected, washed with water and dried over silica gel at 20°. Yield: 13.4 g. (92%). Crystallization from ethanol gave pure 5-iodo-4-hydroxyvaleraldehyde in colourless needles, m.p. 69–70°. (Found: C, 37.4; H, 3.2; I, 43.1. $\text{C}_9\text{H}_8\text{O}_3\text{I}$

requires C, 37.0; H, 3.1; I, 44.5%.) The oxime separated from ethanol in colourless prisms, m.p. 110–111°. (Found: N, 4.4; I, 41.3. $C_9H_{10}O_2NI$ requires N, 4.6; I, 41.3%.)

The 3:5-diiodo-4-hydroxybenzaldehyde was prepared as described by Paal & Mohr (1896).

Biological methods

The compounds were tested in thyroxine-treated mice by the oxygen-consumption test (MacLagan & Sheahan, 1950) with the improvements described by Sheahan, Wilkinson & MacLagan (1951).

RESULTS

The results obtained with a series of ethers and esters of 3:5-diiodo-4-hydroxybenzaldehyde are given in Table 1. With the exception of the carbethoxy derivative and the acetate, which caused significant reductions in the thyroxine effects at total dosages of 200 and 400 mg./kg. respectively, compounds containing the free aldehyde group had little anti-

thyroxine activity. In very high dosage (1000 mg./kg.) the ethyl ether, however, did produce an effect.

The series of acetals also failed to produce any highly active compounds. Apart from the dimethyl acetal of 3:5-diiodoanisaldehyde which, as has already been reported, was active at 1000 mg./kg., the dimethyl acetal of 3:5-diiodo-4-ethoxybenzaldehyde was the only compound which caused any significant diminution in the response to thyroxine (Table 2).

By far the most active members of this series of iodinated aldehydes were found amongst the oximes (Table 3). 3:5-Diiodoanisaldoxime, which was active at a total dose of 50 mg./kg., had a potency only slightly less than that of *n*-butyl 3:5-diiodo-4-hydroxybenzoate, the most active compound we have so far examined. The corresponding ethoxy derivative was also active, though at doses below 400 mg./kg. it was ineffective.

Table 1. *The antithyroxine activity of ethers and esters of 3:5-Diiodo-4-hydroxybenzaldehyde and related compounds*

Compound	Total dosage (mg./kg.) which produced	
	Significant response*	No significant response
R = H	—	400
CH ₃	—	1000
C ₂ H ₅	1000 (74%)	400
	—	400
CH ₃ .CO	400 (67%)	200
C ₂ H ₅ .CO	—	400
<i>n</i> -C ₄ H ₉ .CO	—	400
	—	400
	—	400
	—	400
C ₂ H ₅ O.CO.	200 (24%)	100
	—	400
	—	400
	—	400

* The figures in brackets indicate the percentage inhibition of the thyroxine effect at the stated dose. The figures were subjected to statistical analysis by 'Student's' *t* test.

Table 2. *The thyroxine-inhibitory activity of ethers and esters of 3:5-diiodo-4-hydroxybenzaldehyde dialkyl acetals and similar compounds*

Compound	Total dosage (mg./kg.) which produced	
	Significant response*	No significant response
$\text{RO}-\text{C}_6\text{H}_2(\text{I})_2-\text{CH}(\text{OCH}_3)_2$ $\left\{ \begin{array}{l} \text{R} = \text{CH}_3 \\ \text{C}_2\text{H}_5 \\ \text{C}_6\text{H}_4\text{CH}_2 \\ \text{CH}_2\cdot\text{CO} \\ \text{C}_2\text{H}_5\cdot\text{CO} \end{array} \right.$	1000 (82 %)	400
	1000 (36 %)	400
	—	1000
	—	400
	—	400
$\text{CH}_3\text{O}-\text{C}_6\text{H}_2(\text{I})_2-\text{CH}(\text{OC}_2\text{H}_5)_2$	—	1000
$\text{I}-\text{C}_6\text{H}_2(\text{I})_2-\text{CH}(\text{OCH}_3)_2$	—	400

* See footnote to Table 1.

5-Iodovanillin, 5-iodoveratraldehyde and 5-iodoveratraldioxime were tested as examples of mono-iodo derivatives but none was active at 400 mg./kg. 3:5-Dibromoanisaldehyde and its dimethyl acetal were examined, but were also inactive at this dosage.

Table 3. *The antithyroxine activity of oximes of 3:5-diiodo-4-hydroxybenzaldehyde and related compounds*

Compound	Total dosage (mg./kg.) which produced	
	Significant response*	No significant response
$\text{HO}-\text{C}_6\text{H}_2(\text{I})_2-\text{CH}:\text{NOH}$	—	400
$\text{CH}_3\text{O}-\text{C}_6\text{H}_2(\text{I})_2-\text{CH}:\text{NOH}$	50 (57 %)	25
$\text{C}_2\text{H}_5\text{O}-\text{C}_6\text{H}_2(\text{I})_2-\text{CH}:\text{NOH}$	400 (68 %)	200
$\text{CH}_3\text{O}-\text{C}_6\text{H}_2(\text{I})_2-\text{CH}:\text{NOH}$	—	400

* See footnote to Table 1.

Two ketones, 4-hydroxy- and 4-methoxy-3:5-diiodopropiophenones, were also tested, but at high dosage they proved to be toxic. The maximum tolerated dose of the former was 50 mg./kg., at which it was without effect on the oxygen consumption. The methoxy derivative was rather less toxic and at a total dose of 100 mg./kg. it produced a 36 % inhibition of the thyroxine effect.

DISCUSSION

It is noteworthy that the carbethoxy derivative of 3:5-diiodo-4-hydroxybenzaldehyde was the most active ester of this aldehyde, since, in the preceding paper, it has been shown that, whilst esterification of the phenolic hydroxyl group of *n*-butyl 3:5-diiodo-4-hydroxybenzoate reduced the antithyroxine activity, the carbethoxy derivative exhibited the smallest diminution. In general, however, the aldehydes were much less effective than those series derived from 3:5-diiodo-4-hydroxybenzoic acid.

The high potency of 3:5-diiodoanisaldoxime is in marked contrast to the weak activity of the other derivatives, but in our survey of aldehyde derivatives, it appears that this compound represents a peak since 3:5-diiodo-4-hydroxybenzaldoxime was inactive and the corresponding 4-ethoxy compound showed only a moderate thyroxine-inhibitory action.

SUMMARY

1. A total of twenty-four compounds chemically related to 3:5-diiodo-4-hydroxybenzaldehyde have been examined for antithyroxine activity by the oxygen-consumption method in mice.

2. Of these, 3:5-diiodo-4-ethoxybenzaldehyde and its dimethyl acetal and 3:5-diiodoanisaldehyde dimethyl acetal showed weak activity; the acetyl and carbethoxy derivatives of 3:5-diiodo-4-hydroxybenzaldehyde and 3:5-diiodo-4-ethoxybenzaldoxime were moderately active, whilst 3:5-diiodoanisaldoxime was highly active.

3. Certain mono-iodo compounds related to 5-iodovanillin were also tested, but were without action.

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Comparative Studies of 'Bile Salts'

3. THE CONVERSION OF PYTHOCHOLIC ACID TO DEOXYCHOLIC ACID

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Pythocholic acid, $C_{24}H_{46}O_5$, from the bile of Boidae was shown, as reported in Part 2 of this series (Haslewood & Wootton, 1951), to be a cholanic acid having three secondary hydroxyl groups substituted probably at C-3, C-12 and C-15 or C-16 in the molecule. Such a substance might be convertible to ordinary deoxycholic acid (3 α :12 α -dihydroxy-cholanic acid), and the present report gives details of successful experiments leading to this end. Opportunity is taken to record an investigation of the bile salts of two more species of Boidae.

RESULTS AND DISCUSSION

The dibenzoate (I) of pythocholic lactone was readily obtained. When this was dissolved in warm dilute ethanolic potassium hydroxide, the lactone ring was opened. The resulting acid was precipitated at once and converted into its methyl ester. Without purification, this was oxidized with chromic oxide and a non-crystalline product, which must have consisted chiefly of (II), was isolated. Treatment of this by the Kishner-Wolff method gave deoxycholic acid (III) in a yield of 71%, calculated on the crystalline dibenzoate. Since cholic acid can be converted to deoxycholic acid without change of configuration and in good yield by essentially similar methods (Gallagher & Long, 1943; Haslewood, 1943; Schneider & Hoehn, 1943), the above experiments leave no reasonable doubt that pythocholic lactone is correctly formulated as a 3 α :12 α -dihydroxy compound; pythocholic acid is therefore almost certainly 3 α :12 α :16(or 15)-trihydroxy-cholanic acid.

Results of an examination of the bile salts of two more species of Boidae are given in Table 1. These figures continue to support the view that pythocholic acid is characteristic of the bile of Boidae.

Table 1. *Approximate amounts of bile acids in two species of Boidae*

(Details as described by Haslewood & Wootton, 1951.)

Species	Pythocholic acid (% of 'bile acids'; approx. minimal figures)	Cholic acid (% of 'bile acids'; approx. minimal figures)
Anaconda, <i>Eunicotes murinus</i>	25	8
Reticulated python, <i>Python reticulatus</i>	48	8

EXPERIMENTAL

General. All melting points are uncorrected. Al_2O_3 was obtained from Hopkin and Williams and neutralized as described by Shoppee (1949). Micro-analysis was done by Drs Weiler and Strauss, Oxford. Optical rotations were determined in a 1 dm. micro tube. 20% CrO_3 = a solution made by dissolving CrO_3 (20 g.) in the minimal amount of water and making to 100 ml. with acetic acid.

Conversion of pythocholic lactone to deoxycholic acid

Pythocholic lactone dibenzoate. The lactone (50 mg.) was dissolved in pyridine (0.5 ml.) with benzoyl chloride (0.2 ml.) and the solution heated at about 90° for 30 min. It was cooled and diluted with excess aqueous HCl. Methanol was now added to a concentration of about 60% (v/v) and the solid which gradually formed was collected, washed with approx. 50% (v/v) methanol-water and crystallized from ethanol. Yield, 45 mg., m.p. 198–202°. This substance was