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Note on the Synthesis of the Acetic Acid Analogue of Thyroxine

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The acetic acid analogue of thyroxine has been prepared in order to determine its biological importance; biological experiments will be described in a later paper.

EXPERIMENTAL

Melting points are not corrected for emergent stem.

3:5-*Diiodo*-4-(4′-*methoxyphenoxy*)*benzyl alcohol.* 3:5-Diiodo-4-(4′-methoxyphenoxy)benzaldehyde (Harington & Barger, 1927) (6·5 g.) was boiled under reflux with benzene (15 ml.) and 26 ml. of a solution of aluminium *iso*propoxide in *iso*propanol made from 5·5 g. Al and 200 ml. *iso*propanol. When a test portion of the reaction mixture no longer gave a precipitate with 2:4-dinitrophenylhydrazine, the product was extracted with dilute HCl, and the crystalline residue was collected and crystallized from 70% (v/v) ethanol. The yield was almost quantitative; after a further crystallization from benzene, the compound had m.p. 111–112°. (Found: I, 52·4. $C_{14}H_{12}O_3I_2$ requires I, 52·7%.)

3:5-*Diiodo*-4-(4′-*methoxyphenoxy*)*benzyl chloride.* The above alcohol (3·6 g.) was dissolved in $CHCl_3$ (20 ml.) and cooled in ice-salt, and powdered PCl_5 (1·83 g.) was added in small portions. At the end of the addition, the solution was kept at room temperature for 1 hr., washed with water, dilute $NaHCO_3$ solution, and water again, and dried over $CaCl_2$; on concentrating the solution, the product separated and was collected and crystallized from ethanol. The yield was 3·6 g. (96%). After distillation of a small amount *in vacuo* and crystallization from acetic acid, the compound had m.p. 96·5–97°. (Found: I, 50·7. $C_{14}H_{11}O_2ClI_2$ requires I, 50·8%.)

3:5-*Diiodo*-4-(4′*methoxyphenoxy*)*benzyl cyanide.* The preceding compound (2·25 g.) was dissolved in ethanol (13·5 ml.) containing a trace of KI and boiled under reflux for 4 hr. with KCN (0·45 g.) in water (0·9 ml.); the solution was concentrated to a low volume under diminished pressure and diluted with water, when the product separated. The yield was 2·025 g. (91%). After crystallization from acetic acid, the compound had m.p. 140–142°. (Found: N, 2·7; I, 52·3. $C_{15}H_{11}O_2NI_2$ requires N, 2·9; I, 51·8%.)

3:5-*Diiodo*-4-(4′-*hydroxyphenoxy*)*phenylacetic acid.* The nitrile (1·525 g.) was boiled under reflux for 1 hr. with red P (1 g.) and a mixture of acetic acid (10 ml.) and hydriodic acid, sp.gr. 1·7 (12 ml.). After removal of P the solution was concentrated to dryness, treated with water containing a little bisulphite and concentrated again. The product was dissolved in hot 0·1 N-Na_2CO_3 solution, filtered and acidified at the boiling point with dilute HCl. On cooling, 1·235 g. (80%) of the acid separated; after crystallization from 50% (v/v) acetic acid it had m.p. 214–216·5°. (Found: I, 50·8. $C_{14}H_{10}O_4I_2$ requires I, 51·2%. 9·0 mg. of the acid required 1·74 ml. of 0·0106 N-NaOH for neutralization to phenolphthalein, whence mol.wt. = 490; calc. mol.wt., 496.)

3:5-*Diiodo*-4-(3′:5′-*diiodo*-4′-*hydroxyphenoxy*)*phenylacetic acid.* The iodination offered some difficulty at first, as the conditions used for the preparation of thyroxine from diiodothyronine gave only gummy products which could not be purified; eventually success was achieved by the following method: the diiodo acid (25 mg.) was dissolved in methanol

(1 ml.), and concentrated NH_3 (1 ml.) and cooled in an ice bath; 0·2 ml. of N-I_2 solution was added very slowly with shaking. When the I_2 had all reacted, the solution was diluted with a few ml. of water and most of the NH_3 was removed under diminished pressure at 0°; the solution was then treated with 1 drop of 10% sodium metabisulphite ($Na_2S_2O_5$) solution and acidified at 40° with 3·5 N-HCl. The precipitate which separated was almost entirely crystalline. The yield was 34 mg. (91%). After crystallization from 2 ml. 50% (v/v) methanol, 22 mg. of the compound separated in long needles which had m.p. 219–220° (decomp.). (Found: I, 67·4. $C_{14}H_8O_4I_4$ requires I, 67·9%.)

SUMMARY

The synthesis of 3:5-diiodo-4-(3′:5′-diiodo-4′-hydroxyphenoxy)phenylacetic acid, the acetic acid analogue of thyroxine, is described.

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Some Observations on the Determination of Serum Protein Levels in Cattle

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The copper sulphate specific gravity method of Phillips, Van Slyke, Hamilton, Dole, Emerson & Archibald (1943) has found wide application as a rapid procedure for the determination of serum proteins in man and in certain other species. It has not been adapted to cattle. A recent investigation under field conditions entailed the determination of serum proteins in a large number of zebu cattle and the opportunity was taken to calibrate the specific gravity method against the tyrosine method of Greenberg (1929) which had been adopted as a convenient routine procedure. The Greenberg method has since been standardized on British cattle by comparing the results with those obtained using the usual macro-Kjeldahl technique and, as an independent check, tyrosine was also determined by the spectrophotometric procedure of Goodwin & Morton (1946).

METHODS

Macro-Kjeldahl procedure. Digestions were carried out in triplicate on 2 ml. serum, using the digestion mixture described by Chibnall, Rees & Williams (1943) and heating for at least 8 hr. after the solutions cleared. $Na_2S_2O_3$ (5% w/v) was added to the 40% (w/v) NaOH used to make the digest alkaline (Kantiengar, 1951). Non-protein nitrogen (N.P.N.) was determined by the method of Folin & Wu (1919) with nesslerization. After subtraction of N.P.N., nitrogen was converted to protein using the factor 6·25.

Tyrosine method (Greenberg, 1929). Readings were made in the Hilger-Spekker photoelectric absorptiometer, 30 min. after addition of the Folin-Ciocalteu reagent, against a 200 mg./l. tyrosine solution as standard.

Spectrophotometric determination of tyrosine (Goodwin & Morton, 1946). A 1 in 100 dilution of serum in 0·1 N-NaOH was found to give suitable E values. Readings were made in the Unicam ultraviolet spectrophotometer at 280 and 294·4 mμ. and, to allow correction for irrelevant absorption, at 340 and 370 mμ.

Copper sulphate specific gravity method. The $CuSO_4$ solutions were prepared from a stock solution of $D_{25}^{25} = 1{\cdot}1000$ (King, 1951), the technique described by Phillips *et al.* (1943) being otherwise strictly adhered to.

RESULTS

Tyrosine content of bovine serum proteins. From triplicate determinations on sera from twelve cattle, 1 mg. tyrosine is equivalent to 19·78 ± 1·442 mg. of total protein using the Greenberg method, and 19·68 ± 0·663 mg. of total protein using the technique of Goodwin & Morton. According to Snell & Snell (1937), 1 mg. tyrosine is contained in 19·2 mg. of bovine serum albumin or 20·0 mg. of serum globulin; assuming an albumin : globulin ratio of 2:3 for healthy cattle over 5 years old (Garner, 1950), 1 mg. tyrosine would be equivalent to 19·68 mg. of mixed protein. Greenberg's method (using the equivalent of 0·02 ml. of serum and 0·4 ml. of the standard tyrosine solution) may therefore be adapted to cattle serum by using the expression:

$$\text{Total protein (g./100 ml.)} = \frac{\text{Reading of test } (T)}{\text{Reading of standard } (S)} \times \frac{200 \times 0{\cdot}4}{1000} \times \frac{100}{0{\cdot}02} \times \frac{1}{1000} \times 19{\cdot}73 = \frac{T}{S} \times 7{\cdot}89.$$

The method of Goodwin & Morton would appear to be adaptable to the determination of serum proteins. The amount of irrelevant absorption in a 1 in 100 dilution of serum is negligible in the majority of cases: thus by measuring the extinction