SUMMARY

1. It has been confirmed that the transient fall in adrenal ascorbic acid after the administration of adrenocorticotrophic hormone (ACTH) in the rat does not occur in the chick.

2. A single injection of ACTH resulted in a depletion of adrenal cholesterol in chicks.

3. Evidence has been obtained that in the chick ACTH can stimulate the production of adrenal hormones affecting carbohydrate metabolism although having no effect on adrenal ascorbic acid.

4. The continued administration of ACTH to the chick also caused a retardation of growth, an enlargement of the adrenal and a decreased adrenal cholesterol concentration. Although the ascorbic acid was unaffected in the adrenal there was a fall in its concentration in the blood.

5. No evidence was obtained that endogenous ACTH produced by pituitary stimulants could deplete adrenal ascorbic acid in the chick.

6. Large doses of sodium salicylate caused the ascorbic acid to fall in rat and chicken adrenals and to rise in chicken plasma.

REFERENCES

- Asling, C. W., Reinhardt, W. O. & Li, C. H. (1950). *Endocrinology*, **48**, 534.
- Brodie, B. B., Axelrod, J., Shore, P. A. & Udenfriend, S. (1954). *J. biol. Chem.* **208**, 731.
- Burstein, S., Dorfman, R. I. & Nadel, E. M. (1955). *J. biol. Chem.* **213**, 597.
- Bush, I. E. (1953). *Recent Progr. Hormone Res.* **8**, 505.
- Constable, B. J., Harris, L. J. & Hughes, R. E. (1955). *Brit. J. Nutr.* **10**, 91.
- Coste, F., Bourrel, M. & Delbarre, F. (1953). *C.R. Soc. Biol., Paris*, **147**, 668.
- Cronheim, G., King, J. S. & Hyder, N. (1952). *Proc. Soc. exp. Biol., N.Y.*, **80**, 51.
- Done, A. K., Ely, R. S., Heiselt, L. R. & Kelly, V. C. (1953). *Proc. Soc. exp. Biol., N.Y.*, **83**, 722.
- Golden, W. R. C. & Long, C. N. H. (1942). *Endocrinology*, **30**, 675.
- Good, C. A., Kramer, H. & Somogyi, M. (1933). *J. biol. Chem.* **100**, 485.
- Harris, L. J., Bland, M. N., Hughes, R. E. & Constable, B. J. (1953). *Lancet*, **264**, 1021.
- Harris, L. J., Constable, B. J., Howard, A. N. & Leader, A. (1956). *Brit. J. Nutr.* **10**, 373.
- Jailer, J. W. & Boaz, N. F. (1950). *Endocrinology*, **46**, 314.
- King, E. J. (1951). *Microanalysis in Medical Biochemistry*, 2nd ed., p. 23. London: Churchill.
- Knobil, E., Hagney, M. G., Wilder, E. J. & Briggs, F. N. (1954). *Proc. Soc. exp. Biol., N.Y.*, **87**, 48.
- Long, C. N. H. (1946). *Recent Progr. Hormone Res.* **1**, 101.
- Mindlin, R. C. & Butler, A. M. (1937). *J. biol. Chem.* **122**, 673.
- Olsen, R. E., Jacobs, F. A., Richert, D., Thager, S. A., Kopp, L. J. & Wade, N. S. (1944). *Endocrinology*, **35**, 430.
- Reinecke, R. M. & Kendall, F. C. (1942). *Endocrinology*, **32**, 505.
- Roe, J. H. & Kuether, C. A. (1943). *J. biol. Chem.* **147**, 399.
- Sayers, G. & Sayers, M. A. (1948). *Recent Progr. Hormone Res.* **2**, 81.
- Sayers, G., Sayers, M. A., Lewis, H. L. & Long, C. N. H. (1944). *Proc. Soc. exp. Biol., N.Y.*, **55**, 238.
- Somogyi, M. (1937). *J. biol. Chem.* **117**, 771.
- Stewart, C. P., Horn, D. B. & Redson, J. S. (1953). *Proc. Nutr. Soc.* **12**, 303.
- Taylor, D. J. (1952). *J. clin. Endocrin.* **12**, 822.
- Venning, E. H., Kazmin, V. E. & Bell, J. C. (1946). *Endocrinology*, **38**, 79.
- Weidmann, H. (1955). *Arch. Path. Pharmacol.* **225**, 342.
- Wolfson, W. Q. (1953). *Arch. intern. Med.* **92**, 108.
- Zarrow, M. X. & Baldini, J. T. (1952). *Endocrinology*, **50**, 555.
- Zarrow, M. X. & Zarrow, I. G. (1950). *Anat. Rec.* **108**, 600.
- Zlatkis, A., Zak, B. & Boyle, A. J. (1953). *J. Lab. clin. Med.* **41**, 486.

Studies in the Biochemistry of Micro-organisms

105. CHEMICAL DEGRADATION OF SCLEROTIORIN*

BY J. H. BIRKINSHAW AND P. CHAPLEN

Department of Biochemistry, London School of Hygiene and Tropical Medicine, University of London

(Received 12 December 1957)

Sclerotiorin, a metabolic product originally obtained from the mycelium of *Penicillium sclerotiorum* by Curtin & Reilly (1940), was isolated by us (Birkinshaw, 1952) in considerably larger yield from *Penicillium multicolor*. The molecular formula put forward by Curtin & Reilly was corrected to $C_{21}H_{23}O_5Cl$ and the unsaturated acid obtained on

alkaline hydrolysis was identified as (+)-4:6-dimethylocta-2:4-dienoic acid. This structure for the unsaturated acid was also arrived at independently by Watanabe (1952).

The work was continued and some products resulting from further degradation of sclerotiorin, and particularly of its compound with ammonia, indicating a possible partial structure, were mentioned in a communication (Birkinshaw, 1956) in

* Part 104: Raistrick & Stössl (1958).

which the antibiotic activity of sclerotiorin and of various breakdown products was the main consideration. As it came to our notice that a group of workers at Liverpool (Powell, Robertson & Whalley, 1956) were also investigating sclerotiorin, further work on its constitution was suspended, but it appears appropriate briefly to characterize the degradation products and to describe the method by which they were obtained. Some difficulty was experienced in obtaining crystalline breakdown products of sclerotiorin itself. The product obtained by the action of ammonia on sclerotiorin was examined and proved more amenable to chemical degradation. This compound ($C_{21}H_{24}O_4NCl$), already described (Birkinshaw, 1952) and now for convenience named sclerotiazin, is readily reduced by zinc and acetic acid or by zinc and mineral acid in methanol to a yellow crystalline product sclerotazin ($C_{19}H_{22}O_3NCl$), m.p. 235–237° (decomp.), representing the addition of a molecule of hydrogen and loss of a molecule of acetic acid according to the equation

When hydrochloric acid or sulphuric acid was employed in the reduction the product was isolated as sclerotazin hydrochloride or sulphate respectively. Sclerotazin readily forms a crystalline diacetate, m.p. 150–151°, indicating that the two oxygen atoms are probably present as hydroxyl groups. When sclerotazin is subjected to very mild alkaline conditions a complex reaction seems to occur with formation of mainly amorphous products. One product was obtained in small yield in the form of orange plates, $C_{19}H_{23}O_3N$, m.p. 221–223° (decomp.), in which the chlorine atom of sclerotazin has apparently been replaced by a hydroxyl group.

Ozonolysis converts sclerotiazin diacetate into a crystalline diacetate $C_{15}H_{12}O_6NCl$, m.p. 235–236° (decomp.), which is acidic. This is evidently produced by removal of eight carbon atoms of the unsaturated side chain which gave rise to the dimethyloctadienoic acid produced by alkaline hydrolysis of sclerotiorin. Fission at the $\alpha\beta$ double bond has converted the α -carbon atom of this chain into a carboxyl group present in the new compound, named sclerazinic acid diacetate. The compound is deacetylated by boiling with dilute hydrochloric acid to sclerazinic acid, $C_{11}H_8O_4NCl \cdot H_2O$, the additional molecule of water being presumably water of crystallization. Further evidence of the presence of a carboxyl group in sclerazinic acid was obtained by heating the acid in quinoline with copper chromite catalyst, when one molecular proportion of carbon dioxide was liberated. When sclerazinic acid is oxidized with alkaline permanganate, berberonic acid (I) and acetic acid are formed. In this oxidation three carbon atoms are eliminated in forming the larger fragment. It seems likely that two of these are originally present in a benzene ring fused with the pyridine nucleus at the positions indicated by the two adjacent carboxyl groups located at positions 3 and 4 in berberonic acid, the third carbon atom lost being present as a methyl group attached to this benzene ring and being eliminated in the acetic acid formed in the oxidation. The benzene ring must have as further substituents the two hydroxyl groups and the chlorine atom which have also been lost in the oxidation with permanganate. The penultimate stage in the degradation, sclerazinic acid, should therefore have the structure (II) derived from the diacetate (III). The side chain removed by ozonolysis can

(II) $R_1 = \cdot OH$; $R_2 = \cdot CO_2H$

(III) $R_1 = CH_2 \cdot CO_2$; $R_2 = \cdot CO_2H$

(IV) $R_1 = CH_2 \cdot CO_2$; $R_2 = CH_2 \cdot CH_2 \cdot CHMe \cdot CH \cdot CMe \cdot CH \cdot CH \cdot$

(V) $R_1 = \cdot OH$; $R_2 = CH_2 \cdot CH_2 \cdot CHMe \cdot CH \cdot CMe \cdot CH \cdot CH \cdot$

In (VI), (VII) and (VIII), $R = CH_2 \cdot CH_2 \cdot CHMe \cdot CH \cdot CMe \cdot CH \cdot CH \cdot$

then be located at position 6 of the pyridine ring, adjacent to the nitrogen atom, where the lone carboxyl group stands in berberonic acid. Sclerotiazin diacetate and sclerotazin are therefore accorded structures (IV) and (V) respectively.

The location of the carbon atoms liberated as acetic acid by mild reduction in the conversion of sclerotiazin into sclerotazin is a more difficult problem. The evidence is inadequate to decide this point but a tentative structure (VI) was advanced as a possible solution of the structure of sclerotiazin, leading to structure (VII) for the structure of sclerotiorin.

Compound (VI) represents a type of hemiquinonoid structure such as has been shown to exist in other fungal products, e.g. citrinin and fuscin. Reduction (the addition of 1 mol. of hydrogen) could be expected to transform it into an aromatic (*isoquinoline*) form with loss of acetic acid from the acetate grouping. Tautomeric forms would also be possible.

Methylamine reacts with sclerotiorin in a similar manner to ammonia, replacing one oxygen atom by :NMe to form *N*-methylsclerotiazin, red crystals, m.p. 221°. It was earlier reported (Birkinshaw, 1956) that when this compound was reduced by zinc and acid it forms dihydro-*N*-methylsclerotiazin by addition of two hydrogen atoms without loss of acetic acid. This statement, due to an erroneous analysis, is incorrect. It is now found that the reduction product (yellow needles, m.p. 239–241°), obtained by the action of zinc and acetic acid, has the molecular formula $C_{20}H_{26}O_3NCl$. Deacetylation has therefore occurred in addition to reduction, according to the equation

The deacetylation has been confirmed by the detection of acetic acid as a product when the reduction is performed in methanol in the presence of zinc and sulphuric acid. The acetic acid appears in this case to arise by simple hydrolysis of an acetoxyl group (replacement of acetoxyl by hydroxyl), not, as with sclerotiazin itself, by complete elimination of a molecule of acetic acid (or hydrolysis followed by removal of water) leading to aromatization. This reaction is regarded as lending support to structure (VI) for sclerotiazin. A structure (VIII) for sclerotiorin, somewhat similar to (VII), differing considerably, however, in the method of attachment of the two carbon atoms giving rise to acetic acid, was proposed by Powell *et al.* (1956).

EXPERIMENTAL

All melting points are uncorrected. Elementary analyses are by Weiler and Strauss, Oxford, or Imperial Chemical

Industries Ltd., Akers Research Laboratories, Welwyn, Herts.

Light-absorption maxima of sclerotiorin and sclerotiazin. Sclerotiorin: 227, 287, 362, 440–450 (inflection) $m\mu$ in ethanol; $\log \epsilon$ 4.20, 4.24, 4.60, 4.20 respectively. Sclerotiazin: 235, 332, 500 $m\mu$ in ethanol; $\log \epsilon$ 4.10, 4.68, 3.68 respectively.

Reduction of sclerotiazin with zinc-acetic acid. Production of sclerotazin. Sclerotiazin (1.0 g.) was stirred with Zn dust (1.0 g.) and acetic acid (50 ml.). The colour of the solution rapidly changed from intense red to yellow-orange. All the sclerotiazin dissolved but some Zn remained and some zinc acetate crystallized out. When there was no further colour change (15 min.) the mixture was filtered and the residue on the filter was washed with acetic acid. The filtrate was poured into water (250 ml.) and the bright-yellow ppt. was allowed to settle, then collected and dried (wt. 1.0 g.). Recrystallization from methanol afforded golden-yellow prisms (double pyramids) of *sclerotazin*, m.p. 235–237° (decomp.). Light-absorption max. 223, 295, 410, 475 (inflection) $m\mu$ in ethanol; $\log \epsilon$, 4.27, 4.61, 3.59, 2.59 respectively. The same product was obtained when propionic acid was substituted for acetic acid as solvent (Found: C, 68.9, 69.1, 68.95; H, 7.0, 6.8, 6.65; N, 4.2, 4.2, 4.35; Cl, 10.4, 10.7. $C_{19}H_{22}O_2NCl$ requires C, 68.8; H, 6.7; N, 4.2; Cl, 10.7).

Reduction of sclerotiazin with zinc-sulphuric acid in methanol. Production of sclerotazin sulphate. Sclerotiazin (1.0 g.) and Zn dust (1.0 g.) in methanol (50 ml.) were stirred and H_2SO_4 (50%, v/v; 1 ml.) was added to the mixture. The colour quickly changed through orange to golden-yellow (10 min.). The liquid was decanted into water (250 ml.), leaving the undissolved Zn. The bright-yellow flocculent ppt. was collected, washed with water and dried (wt. 0.97 g.). Recrystallization from methanol afforded lemon-yellow plates of *sclerotazin sulphate*, m.p. unsharp, mainly 174–178° after softening from 170° (Found: C, 57.3, 57.45; H, 6.3, 6.3; N, 3.7, 3.5; Cl, 10.2, 10.2; S, 4.2. $2C_{19}H_{22}O_2NCl \cdot H_2SO_4 \cdot 2H_2O$ requires C, 57.3; H, 6.3; N, 3.5; Cl, 8.9; S, 4.0%).

The aqueous filtrate from the sclerotazin sulphate was evaporated *in vacuo* almost to dryness and the evaporation was repeated twice after addition of water (50 ml. each time) to the residue. The distillates on titration were found to contain $18.77 + 0.43 + 0.11$ ml. (total 19.3 ml.) of 0.1 *N*-volatile acid (75% of 1 mol. prop.). The neutralized distillates were concentrated to about 3 ml. and *p*-bromophenacyl bromide (0.54 g.) and ethanol (5 ml.) were added. The solution was refluxed for 1.5 hr. Addition of water to the warm solution to incipient turbidity produced crystals (0.43 g.) of m.p. 82–83°, raised on recrystallization to 85–86°. Admixture with authentic *p*-bromophenacyl acetate of the same m.p. produced no depression. Thus acetic acid is a product of the reaction.

Reduction of sclerotiazin with zinc-hydrochloric acid in methanol. Formation of sclerotazin hydrochloride. The reaction was carried out as with H_2SO_4 , with 2.0 ml. of conc. HCl in place of 1.0 ml. of 50% H_2SO_4 . The product, a yellow powder, when crystallized from butan-1-ol, gave *sclerotazin hydrochloride* of m.p. 256–260° (decomp.) shrinking from 250° (Found: C, 61.4; H, 6.25; N, 3.5; Cl, 19.5. $C_{19}H_{22}O_2NCl \cdot HCl$ requires C, 61.9; H, 6.3; N, 3.8; Cl, 19.35%).

Di-O-acetylsclerotazin. Sclerotazin (0.1 g.), anhydrous sodium acetate (0.2 g.) and acetic anhydride (1.0 ml.) were

heated together at 140° (bath temp.) for 30 min. The cooled mixture was poured into water and the cream-coloured solid (0.12 g.) so obtained was recrystallized from methanol, affording colourless needles of *sclerotazin diacetate*, m.p. 150–151°. The same product was obtained in similar yield by acetylation of sclerotazin sulphate by the same method (Found: C, 66.35, 66.5, 66.55; H, 6.4, 6.3, 6.3; N, 3.5, 3.5; Cl, 8.6, 8.7. $C_{23}H_{26}O_4NCl$ requires C, 66.4; H, 6.3; N, 3.4; Cl, 8.5%).

Mild alkaline hydrolysis of sclerotazin. Replacement of Cl by OH. (i) With NaOH. Sclerotazin (0.72 g.) was dissolved by warming in ethanol (60 ml.) and to the warm solution was added *n*-NaOH (2.2 ml., 1 equiv.). The solution, which rapidly darkened, was kept at room temp. for 18 hr. and then the ethanol was removed under reduced pressure. The residue was completely soluble in water. The intensely coloured aqueous solution was extracted with ether in a continuous extractor for 6 hr. On concentration of the ether solution to about 20 ml. a deep-red amorphous material (0.03 g.) separated and was removed. The ether was completely evaporated from the filtrate. The residue (0.39 g.) was crystallized from methanol, which afforded 70 mg. of orange plates with a bronzy lustre of *hydroxy-dechlorosclerotazin*, m.p. 221–223° (decomp.) (Found: C, 72.7; H, 6.8; N, 4.4; Cl, nil. $C_{19}H_{23}O_3N$ requires C, 72.8; H, 7.2; N, 4.5%). Further evaporation of the mother liquor afforded more orange plates and some double pyramids characteristic of sclerotazin. Only a small amount of amorphous ppt. (0.08 g.) was obtained from the ether-extracted aqueous solution on acidification.

(ii) With ethanolic potassium acetate. Sclerotazin sulphate (1 g.) dissolved in warm ethanol (150 ml.) was treated with potassium acetate in ethanol (50 ml. of 5% w/v, solution). The mixture darkened and the deep-red solution gradually deposited colourless prisms (0.10 g.) of potassium chloride. After 10 days the solution was concentrated under reduced pressure to about 50 ml. and water (200 ml.) was added. Treatment of the emulsion obtained with 2*N*- H_2SO_4 (15 ml.) precipitated a dark-red solid (0.73 g.), from which by crystallization from methanol gleaming orange plates (0.05 g.) of m.p. 220° (decomp.) were obtained. This was the same product, hydroxydechlorosclerotazin, as that given by method (i) (Found: C, 72.7; H, 6.6; N, 4.5%; Cl, nil).

Ozonolysis of diacetate: sclerazinic acid diacetate. Sclerotazin diacetate (3.6 g.) dissolved in $CHCl_3$ (120 ml.) was treated with ozonized oxygen for 2.5 hr. The solution, yellow at first, slowly became almost colourless and towards the end of the period a small amount of flocculent ppt. appeared. The ozonized product was decomposed by shaking the solution with water (120 ml.) during 0.5 hr. An almost colourless solid (0.52 g.), m.p. 235–236° (decomp.) separated and was collected. The aqueous layer was discarded and the $CHCl_3$ layer was concentrated. Further fractions of the same solid were obtained (total wt. 1.88 g.). The final residue, an orange-red oil, was not further examined. Crystallization of the solid from ethyl malonate gave pale-cream microneedles of *sclerazinic acid diacetate* of m.p. 235–236° (decomp.) (Found: C, 53.25, 53.1; H, 3.75, 3.8; N, 4.0, 3.9; Cl, 10.5, 10.5. $C_{15}H_{12}O_6NCl$ requires C, 53.35; H, 3.6; N, 4.15; Cl, 10.5). The product is acidic since it dissolves in aqueous bicarbonate.

Sclerazinic acid. Sclerazinic acid diacetate (1.65 g.) was deacetylated by refluxing with *n*-HCl (400 ml.) until all the

solid dissolved (1.5 hr.). The cooled solution was brought to pH 4.8–5.0 with 2*N*-NaOH. A lime-yellow ppt. slowly formed. The pptn. was completed at 0° and the product, *sclerazinic acid*, was collected (wt. 1.25 g.). It did not melt up to 360° but slowly darkened to a black pellet; light absorption max. 267, 354 $m\mu$ in ethanol; $\log \epsilon$ 4.56, 3.62 respectively (Found: on material dried at 80°, C, 48.5, 48.7; H, 3.6, 3.75; N, 5.0, 5.0; Cl, 12.8, 13.0; loss in weight at 120°, 4.7. $C_{11}H_8O_4NCl \cdot H_2O$ requires C, 48.6; H, 3.7; N, 5.2; Cl, 13.05; H_2O , 6.6%).

Sclerazinic acid hydrochloride. The diacetate (1 g.) was boiled under reflux with *n*-HCl (250 ml.) until all dissolved. Crystalline nodules separating were collected after 2 days and washed on the filter with dil. HCl. The product, *sclerazinic acid hydrochloride*, had m.p. not below 360°. The filtrate deposited a small amount of sclerazinic acid on adjusting to pH 5 (Found: C, 43.9; H, 4.5; N, 4.2; Cl, 20.1. $C_{11}H_8O_4NCl \cdot HCl \cdot H_2O$ requires C, 42.9; H, 3.6; N, 4.55; Cl, 23.0%). The substance has evidently lost some HCl on drying.

Decarboxylation of sclerazinic acid. Sclerazinic acid hydrochloride (0.504 g.), quinoline (7 ml.) and copper chromite catalyst (0.2 g.) were heated in a closed system with an air condenser. A current of CO_2 -free N_2 was passed during the heating at bath temp. 160–170° for 3 hr. and at 220–230° for 3 hr. The issuing gases were bubbled through standard aq. $Ba(OH)_2$ soln. During the experiment CO_2 equivalent to 12.77 ml. of 0.25*N*- $Ba(OH)_2$ was obtained, only about 10% of which was released at the lower temp. Assuming a mol.wt. of 308 for the hydrochloride, this represents 98% of one mol.prop. of CO_2 . No crystalline product could be recovered from the quinoline.

Oxidation of sclerazinic acid with potassium permanganate: isolation of berberonic acid. Sclerazinic acid (0.51 g.) was dissolved by shaking in water (50 ml.) with a little $NaHCO_3$. Powdered $KMnO_4$ was slowly added with shaking until the colour was no longer discharged in 1 hr. The hydrated MnO_2 was removed by filtration and to the filtrate *n*- H_2SO_4 was added until it was just acid to Congo red. The colour darkened to orange. The solution was evaporated *in vacuo* nearly to dryness four times, water being added before the distillations subsequent to the first. The volatile acid amounted to 11.24 ml. of 0.1*N* (60% of 1 mol. prop.). This was converted into the *p*-bromophenacyl ester in the usual manner. Crystals of m.p. 85–86° were obtained, which did not depress the m.p. of authentic *p*-bromophenacyl acetate. Therefore the volatile acid produced in the reaction is acetic acid.

The non-volatile aqueous residue was extracted with ether in a continuous extractor for 24 hr. The crystalline solid obtained (0.28 g.) on removal of the ether had m.p. 233° (decomp., frothing). Recrystallization from water (decolorizing charcoal) gave compact prisms, m.p. 238–240° (decomp.). The product contained no chlorine, gave with $FeCl_3$ a white or very light-yellow ppt. and with $FeSO_4$ a port-wine colour (Found: C, 42.1; H, 3.5; N, 6.05. $C_8H_8O_6N \cdot H_2O$ requires C, 41.9; H, 3.1; N, 6.1%). A sample of berberonic acid prepared from berberine nitrate by oxidation with nitric acid, when recrystallized from water had m.p. 241–242° (decomp.), and there was no depression on admixture with the product of oxidation with $KMnO_4$. Hence the latter is berberonic acid.

For confirmation the *p*-bromophenacyl esters of the two acids were prepared. The ester from the product of oxid-

ation with KMnO_4 had m.p. 175–176° after recrystallization from ethanol (microrosettes of needles) (Found: C, 48.3; H, 2.7; N, 1.85; Br, 29.1. $\text{C}_{22}\text{H}_{20}\text{O}_3\text{NBr}_2$ requires C, 47.9; H, 2.5; N, 1.75; Br, 29.9%). The *p*-bromophenacyl ester of authentic berberonic acid had the same m.p., 175–176°, and there was no depression on mixing.

Action of methylamine on sclerotiorin: *N*-methylsclerotiorazin. Sclerotiorin (0.25 g.) was treated with aqueous methylamine (33%, w/v; 5 ml.). The substance immediately became dark red but complete solution was not attained. The mixture was well stirred and all lumps were broken up. After 15 min., water (25 ml.) was added, the mixture was made just acid to Congo red and the solid (0.22 g.) was collected. Crystallization from aq. methanol (50%, v/v) furnished an *N*-methylsclerotiorazin as purple-black needles, m.p. 220–222° (Found: C, 65.3; H, 6.3; N, 3.8; Cl, 8.8. $\text{C}_{22}\text{H}_{26}\text{O}_4\text{NCl}$ requires C, 65.4; H, 6.5; N, 3.5; Cl, 8.8%).

Reduction of N-methylsclerotiorazin. *N*-Methylsclerotiorazin (0.93 g.) in acetic acid (40 ml.) was well stirred during the addition of Zn dust (1 g.). The colour of the solution changed from deep red to orange. The reaction was considered complete when no further lightening occurred (about 20 min.). The solution was filtered to remove residual Zn and poured into water. The gelatinous ppt. produced gradually crystallized in sheaves of needles. The product (0.74 g., m.p. 239°) formed golden-yellow needles of *deacetyldihydro-N*-methylsclerotiorazin, m.p. 239–241°, on recrystallization from methanol (Found: C, 65.9, 66.0; H, 7.4, 7.3; N, 3.5; Cl, 9.85, 9.9. $\text{C}_{20}\text{H}_{26}\text{O}_3\text{NCl}$ requires C, 66.0; H, 7.2; N, 3.85; Cl, 9.7%).

N-Methylsclerotiorazin (2 g.) was dissolved in methanol and reduced in presence of Zn dust (2 g.) and H_2SO_4 (50%, v/v; 2 ml.). The product was poured into water and the filtrate was distilled in the usual manner for collection of volatile acids. Titration of the distillate revealed that 46.8 ml. of 0.1*N*-volatile acid had been produced (94% of 1 mol.prop.). Conversion into the *p*-bromophenacyl ester afforded a product of m.p. 84–85°, not depressed on admixture with *p*-bromophenacyl acetate.

SUMMARY

1. The product of the reaction of sclerotiorin with ammonia, $\text{C}_{21}\text{H}_{24}\text{O}_4\text{NCl}$, named sclerotiorazin, is readily reduced by zinc and acetic acid, yielding sclerotazin ($\text{C}_{19}\text{H}_{22}\text{O}_3\text{NCl}$) and acetic acid.

2. By mild alkaline hydrolysis of sclerotazin the chlorine atom is eliminated and replaced by a hydroxyl group, affording hydroxydechlorosclerotazin, $\text{C}_{19}\text{H}_{23}\text{O}_3\text{N}$.

3. Sclerotazin forms a diacetate, from which the unsaturated side chain is removed by ozonolysis and replaced by a carboxyl group, affording the diacetate of sclerazinic acid $\text{C}_{11}\text{H}_8\text{O}_4\text{NCl}$.

4. Oxidation of sclerazinic acid with alkaline potassium permanganate solution gives berberonic acid. From these degradations a tentative partial molecular structure for sclerotazin is deduced.

5. Sclerotiorin reacting with methylamine produces *N*-methylsclerotiorazin ($\text{C}_{22}\text{H}_{26}\text{O}_4\text{NCl}$), which is reduced by zinc and acetic acid to deacetyldihydro-*N*-methylsclerotiorazin, $\text{C}_{20}\text{H}_{26}\text{O}_3\text{NCl}$.

We wish to thank Imperial Chemical Industries Ltd., Akers Research Laboratories, The Frythe, Welwyn, Herts, for carrying out some of the elementary analyses. The Uvispek Spectrophotometer used in this work was purchased by means of a grant from the Central Research Fund of London University.

REFERENCES

- Birkinshaw, J. H. (1952). *Biochem. J.* **52**, 283.
 Birkinshaw, J. H. (1956). *G. Microbiol.* **2**, 116.
 Curtin, T. P. & Reilly, J. (1940). *Biochem. J.* **34**, 1419.
 Powell, A. D. G., Robertson, A. & Whalley, W. B. (1956). *Spec. Publ. Chem. Soc.* **5**, 27.
 Raistrick, H. & Stössl, A. (1958). *Biochem. J.* **68**, 647.
 Watanabe, H. (1952). *J. pharm. Soc. Japan*, **72**, 807.

Comparative Detoxication

5. CONJUGATION OF AROMATIC ACIDS IN REPTILES: FORMATION OF ORNITHURIC ACID, HIPPURIC ACID AND GLUCURONIDES*

By J. N. SMITH

Department of Biochemistry, St Mary's Hospital Medical School, London, W. 2

(Received 28 January 1958)

The detoxication of aromatic acids in the animal body may involve condensation with a variety of compounds in different species and, as a rule, more than one type of conjugation is found in each species. Glycine conjugation occurs in most mammals (Williams, 1947), in frogs (Bunge &

Schmiedeberg, 1876; Komori, Sendju, Sagara & Takamatsu, 1926), in some birds (Wu Chang & Johnson, 1957) and in insects (Smith, 1955). Ester glucuronides can also be formed in mammals (Williams, 1947) and in the chick (Wu Chang & Johnson, 1957; B. C. Baldwin, D. Robinson & R. T. Williams, unpublished work). Glutamine conjugations, which have been thought peculiar to

* Part 4: Smith (1955).