The Biochemistry of Aromatic Amines

5. 2-ACETAMIDO-6-HYDROXY-5-NAPHTHYL HYDROGEN SULPHATE AND OTHER METABOLITES OF 2-NAPHTHYLAMINE AND 2-ACETAMIDONAPHTHALENE*

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Exposure to 2-naphthylamine is known to cause cancer of the bladder, but not of any other organ in man. For this and other reasons the carcinogenic effect is thought to be exerted through some metabolite excreted in the urine. The metabolism of this and other aromatic amines has therefore been intensively studied, and a list of the known metabolites of 2-naphthylamine is given in Table 1. 2-Acetamido-6-hydroxy-5-naphthyl hydrogen sulphate (described in the present paper) has been isolated as a metabolite of 2-acetamidonaphthalene and detected as a metabolite of 2-naphthylamine.

EXPERIMENTAL

Methods

Animals. Animals were kept in metabolism cages and the urine was collected daily. Rabbits (average wt. 2 kg.) were fed on cabbage, bran and water, and rats on rat cake, bread and water. Rabbits were injected intraperitoneally with 0·5 g. of 2-naphthylamine or 0·5 g. of 2-acetamidonaphthalene in 10 ml. of arachis oil. Rats (average wt. 200 g.) were dosed in the same way with 0·05 g. of either compound in 1 ml. of oil.

Paper chromatography. Whatman no. 1 paper was employed with the following solvent systems: (*a*) butanol–propan-1-ol–aq. 0·1 N-NH_3 soln. (2:1:1, by vol.), (*b*) butanol–acetic acid–water (2:1:1, by vol.) and (*c*) butanol–acetic acid–water (70:5:19, by vol.) and Whatman no. 3 MM paper was used with the solvent system (*d*) butanol–acetic acid–water (10:1:1, by vol.).

Reagents used in this work and R_F values of some 2-naphthylamine derivatives have been described previously (Booth, Boyland & Manson, 1955). Acetamido compounds and hydrogen sulphates were hydrolysed on chromatograms by spraying the paper with N-HCl and heating between glass sheets for 0·5 hr. at 70° in an oven. A Hanovia Chromatolite was used as a source of ultraviolet light. 2-Amino-1-naphthol hydrochloride could not be run on chromatograms without oxidation to give coloured compounds at the solvent front and along the line of flow. The use of a solvent system containing HCl (Ekman, 1948) did not prevent this. If the paper was pretreated by spraying with ascorbic acid (1% in water) until just moist and then dried with gentle heat, 2-amino-1-naphthol hydrochloride could then be run in solvent system (*b*)

* Part 4: Boyland & Manson (1957).

Table 1. *Metabolites of 2-naphthylamine*

References: 1, Wiley, 1938; 2, Dobriner *et al.* 1941; 3, Boyland & Manson, 1957; 4, Boyland & Manson, present work; 5, Boyland *et al.* 1957; 6, Manson & Young, 1950.

Compound	Reference to Identification	Reference to Isolation
2-Acetamidonaphthalene	—	2, 4
2-Naphthylsulphamic acid	—	5
2-Naphthylamine *N*-glucosiduronic acid	—	5
2-Amino-1-naphthyl hydrogen sulphate	—	1, 5, 6
2-Amino-1-naphthyl glucosiduronic acid	3	—
2-Amino-1-naphthyl hydrogen sulphate *N*-glucosiduronic acid	5	—
2-Amino-6-naphthol	2, 4	—
2-Amino-6-naphthyl hydrogen sulphate	4	—
2-Amino-6-naphthyl glucosiduronic acid	3	—
2-Acetamido-6-naphthol	—	2, 6, 4
2-Acetamido-6-naphthyl hydrogen sulphate	4	—
2-Acetamido-6-naphthyl glucosiduronic acid	—	3
2-Acetamido-6-hydroxy-5-naphthyl hydrogen sulphate	—	4
2-Acetamido-5:6-dihydro-5:6-dihydroxynaphthalene	4	—
2-Acetamido-5:6-dihydro-5:6-dihydroxynaphthalene glucosiduronic acid	3	—

(R_F 0·85) without much oxidation (cf. paper chromatography of adrenaline and noradrenaline, Crawford & Outschoorn, 1951). The aminonaphthol could then be detected by Ehrlich's reagent or by the characteristic bluish-purple colour on exposure to NH_3 vapour. Aqueous solutions of 2-amino-1-naphthol hydrochloride give a green colour on the addition of aq. NH_3 soln. or other alkali. If the solution is then shaken with benzene, the organic phase becomes purple (Liebermann & Jacobson, 1882).

Urine was applied directly to the chromatograms or concentrated before application by adsorption on charcoal deactivated with stearic acid (7·5 % in ethanol) (Asatoor & Dalgliesh, 1956). Urine (10 ml.) was passed under suction through a charcoal column (2·0 cm. × 3·5 cm.) and the column was then washed with water (3 × 25 ml.) and aq. phenol soln. (5 %, 100 ml.). The phenol eluate was evaporated to dryness under reduced pressure. Residual phenol was removed in a desiccator over NaOH.

Materials

All analytical samples were dried at 0·5 mm. Hg and 100° for 2 hr. Most of the compounds used have been described previously (Booth *et al.* 1955). 2-Diacetamidonaphthalene was prepared by the method of Sudborough (1901).

2-*Acetamido*-5:6-*diacetoxynaphthalene*. 6-Acetamido-1:2-naphthaquinone (Kehrmann & Matis, 1898) was reduced to the corresponding dihydroxy compound by the method of Fieser & Fieser (1939) for the reduction of 1:2-naphthaquinone. The finely powdered quinone (0·7 g.) was added in portions over 15 min. to $Na_2S_2O_4$ (1·4 g.) in water (20 ml.) with shaking and slight warming on the water bath. The quinone decolorized rapidly and the product remained mainly in suspension. After cooling, the buff-coloured solid (0·6 g.) was collected, washed with water and dried. The product was not purified, but it gave one spot (R_F 0·9) on an ascending chromatogram developed in solvent system (*b*). The spot was faintly fluorescent, became greenish brown on exposure to light and reddish blue on spraying with dilute Na_2CO_3 soln. but gave no additional colour with diazotized sulphanilic acid. After hydrolysis on the chromatogram the spot gave an orange colour with Ehrlich's reagent. The compound could not be run in solvent system (*a*) without the formation of coloured products. The dihydroxy compound was kept overnight in pyridine (2 ml.) and acetic anhydride (2 ml.) at room temperature. The solution was poured into water and the precipitate collected and crystallized from aq. ethanol to yield 2-*acetamido*-5:6-*diacetoxynaphthalene* (plates), m.p. 172–174° (Found: C, 64·1; H, 5·1; N, 4·4. $C_{16}H_{15}O_5N$ requires C, 63·8; H, 5·0; N, 4·65 %).

Potassium 2-*acetamido*-6-*hydroxy*-5-*naphthyl sulphate*. 6-Nitro-2-naphthylamine was prepared from 2-naphthylamine (Saunders & Hamilton, 1932) and converted into 2-nitro-6-naphthol (Veselý & Jakes, 1923). A saturated aq. solution of $K_2S_2O_8$ (7 g.) was added with stirring to 2-nitro-6-naphthol (5 g.) and KOH (5 g.) in water (100 ml.) over 4 hr. After being kept overnight at room temperature, the solution was adjusted to pH 5·0 and extracted several times with ether. The aqueous phase was made alkaline again with KOH and evaporated to dryness under reduced pressure. The residue was crystallized from aq. ethanol to give *dipotassium* 2-*nitro*-6-*hydroxy*-5-*naphthyl sulphate* (1·6 g.), as red needles blackening at 200° but not melting below 360° (Found: C, 31·5; H, 2·1; N, 3·7; S, 8·1; K, 20·4. $C_{10}H_5O_7NSK_2,H_2O$ requires C, 31·65; H, 1·9; N, 3·5; S, 8·4; K, 20·6 %). The nitro compound (0·7 g.) was shaken with hydrogen and Adam's catalyst in aq. ethanol. After filtration the solution was evaporated to dryness under reduced pressure. The solution darkened considerably. The residue was acetylated with thioacetic acid (0·4 g.) in pyridine (10 ml.) (cf. Booth *et al.* 1955). After being kept at room temperature overnight, the solution was evaporated to dryness in a desiccator over P_2O_5 and NaOH. The residue was crystallized from aq. ethanol to give *potassium*-2-*acetamido*-6-*hydroxy*-5-*naphthyl sulphate*, m.p. 185–188° (decomp.) (Found: C, 41·0; H, 3·4; N, 3·9; S, 8·6; K, 10·8. $C_{12}H_{10}O_6NSK,H_2O$ requires C, 40·8; H, 3·4; N, 4·0; S, 9·1; K, 11·1 %). On paper chromatograms the compound had R_F values of 0·45 (descending) and 0·5 (ascending) in solvent system (*a*) and 0·57 (ascending) in solvent system (*b*). The spot became greenish brown upon exposure to daylight. It gave a pale-pink colour on spraying with Na_2CO_3 and diazotized sulphanilic acid solutions, but did not diazotize and couple with hexylresorcinol. After acid treatment of the chromatogram at 70° the spot diazotized (greenish-yellow colour), but on spraying with alkaline hexylresorcinol it gave the same dirty red colour as it gave on spraying with alkali alone without diazotization. After hydrolysis on the chromatogram the spot gave an orange colour with Ehrlich's reagent. After hydrolysis in solution it gave a red colour on addition of alkali, changing to dirty green with shaking. On extraction with benzene, the organic phase was purple but faded after 30 min. The last colour reaction resembles that of 2-amino-1-naphthol except that with this compound the benzene has a darker red tinge and is stable for several hours. Potassium 2-acetamido-6-hydroxy-5-naphthyl sulphate was not hydrolysed by the sulphatase of Taka-diastase (Parke, Davis and Co. Ltd.).

The triacetyl derivative of 2-amino-5:6-dihydroxynaphthalene was prepared as follows. The nitro compound (0·6 g.) was reduced as before and the solution evaporated to dryness under reduced pressure. The residue was dissolved in water and acidified with conc. HCl to precipitate 2-amino-6-hydroxy-5-naphthyl hydrogen sulphate. This compound was not purified. It gave a mauve colour in alkaline solution with diazotized sulphanilic acid and a bluish-red colour on diazotization and coupling with hexylresorcinol. It had R_F values of 0·32 (ascending), with a green-coloured tail, in solvent system (*a*) and 0·5 (ascending), with tailing, in solvent system (*b*). The compound was hydrolysed by heating with 5 N-HCl at 100° for 30 min. The solution was cooled and the 2-amino-5:6-dihydroxynaphthalene collected, and acetylated with acetic anhydride in pyridine. The product was crystallized from aq. ethanol to yield plates, m.p. 173–174°, which did not depress the melting point of 2-acetamido-5:6-diacetoxynaphthalene prepared from the reduction of 6-acetamido-1:2-naphthaquinone. Attempts to prepare 2-acetamido-6-hydroxy-5-naphthyl hydrogen sulphate by treating 2-acetamido-6-naphthol with alkaline persulphate were unsuccessful and none was detected in the reaction mixture by paper chromatography.

RESULTS

Isolation of metabolites

2-*Naphthylamine*, 2-*acetamidonaphthalene and* 2-*acetamido*-6-*naphthol*. 2-Naphthylamine (0·5 g. in 10 ml. of arachis oil) was injected intraperitoneally into each of five

rabbits for 11 days (total 27·5 g.) and the urine collected daily. The urine was adjusted to pH 6·5 and continuously extracted with ether for 16 hr. The ether extract was washed several times with 2 N-HCl. The washings were made alkaline and the precipitate was collected and crystallized from aq. ethanol to give 2-naphthylamine (0·6 g., 2 % of the dose), m.p. and mixed m.p. 109–111° (Found: N, 9·6. Calc. for $C_{10}H_9N$: N, 9·8 %). The ether extracts were evaporated to small bulk and cooled to give crude 2-acetamido-6-naphthol (3·5 g.), m.p. 190–199°. Two crystallizations from aq. ethanol gave 1 g. (equivalent to 2 % of the dose) of the acetamidonaphthol, m.p. and mixed m.p. 220–222° (Found: C, 71·9; H, 5·8; N, 6·95. Calc. for $C_{12}H_{11}O_2N$: C, 71·6; H, 5·5; N, 6·9 %). The ethereal solution was diluted with more ether and washed with 2 N-NaOH to remove residual 2-acetamido-6-naphthol, followed by water, and then dried and evaporated to dryness. The residue was extracted several times with hot light petroleum (b.p. 80–100°) and the combined extracts were evaporated to dryness. Crystallization of the residue from aq. ethanol gave 2-acetamidonaphthalene (3 mg.), m.p. and mixed m.p. 129–131°. After rabbits had been injected with 2-acetamidonaphthalene, 0·2 g. of 2-acetamido-6-naphthol was isolated from a total dose of 16·8 and 0·8 g. from a total dose of 11·5 g. These yields were equivalent to 1 and 6 % of the administered material. Dobriner, Hoffman & Rhoads (1941) isolated these compounds, in unstated yields, from rats, rabbits and monkeys dosed with 2-naphthylamine. Manson & Young (1950) isolated 2-acetamido-6-naphthol from rats injected with 2-naphthylamine and 2-acetamidonaphthalene, and recovered 2-naphthylamine and 2-acetamidonaphthalene from the urine of rats injected with these compounds.

2-*Acetamido*-6-*hydroxy*-5-*naphthyl hydrogen sulphate*. In the isolation of 2-acetamido-6-naphthyl glucosiduronic acid from rabbits injected with 28 g. of 2-acetamidonaphthalene a new compound was encountered on separating the metabolites on a cellulose column (Boyland & Manson, 1957, p. 277). From its behaviour in solvent system (*a*) it appeared to be a hydrogen sulphate. The fraction eluted from the cellulose column contained both this and 2-acetamido-6-naphthyl hydrogen sulphate. The eluate was made alkaline with aq. KOH soln. and evaporated to 5 ml. After cooling at 5° for 5 days, crystals separated which were collected and recrystallized from aq. ethanol to yield plates (0·09 g.) of potassium 2-acetamido-6-hydroxy-5-naphthyl sulphate, m.p. and mixed m.p. 188–189° (decomp.) (Found: C, 40·8; H, 3·5; N, 4·2; S, 9·0; K, 11·0. Calc. for $C_{12}H_{10}O_6NSK,H_2O$: C, 40·8; H, 3·4; N, 4·0; S, 9·1; K, 11·1 %). The infrared spectrum was identical with that of the synthetic ester. The compound gave a precipitate on heating with $BaCl_2$ in 2 N-HCl. The colour reactions and R_F values were identical with those of the synthetic compound and it showed the same resistance to hydrolysis by Taka-diastase. The compound was not easily detected in untreated urine by one-dimensional paper chromatography in solvent system (*a*) as the spot was almost in the same position as indoxyl hydrogen sulphate and 2-acetamido-6-naphthyl hydrogen sulphate. It was more readily detected after adsorption of the metabolites on charcoal followed by elution with phenol. The residue from the eluate was examined by two-dimensional paper chromatography in solvent system (*a*) followed by (*b*). The spot was then detected by the colour formed on exposure to light for several hours. The metabolite was also detected in the urine of rabbits and rats dosed with 2-naphthylamine. In solvent system (*d*) the compound had the same R_F (0·4, descending) as 2-acetamido-6-naphthyl hydrogen sulphate and indoxyl hydrogen sulphate. The mother-liquors from the isolation of the metabolite contained 2-acetamido-6-naphthyl hydrogen sulphate, identified by its colour reactions and by the release of 2-acetamido-6-naphthol on incubation with Taka-diastase.

Paper chromatography of urine after injection of 2-naphthylamine and 2-acetamidonaphthalene

Rats and rabbits were injected with 2-naphthylamine and the urine was examined for other metabolites than those already described by Boyland, Manson & Orr (1957) and Boyland & Manson (1957). 2-Naphthylamine, 2-amino-1-naphthyl hydrogen sulphate and 2-acetamido-6-naphthol were identified in the urine of both species. 2-Amino-6-naphthol and its hydrogen sulphate were found in rat urine, although not consistently, but never in rabbit urine. For the identification of 2-acetamidonaphthalene solvent system (*c*) was used. In this system 2-naphthylamine had an R_F value of 0·90 and the acetamido derivative one of 0·95. In the urine of both species a non-fluorescent spot was found which agreed with the R_F value of 2-acetamidonaphthalene and which diazotized and coupled only after acid treatment of the chromatograms. 2-Diacetamidonaphthalene, however, behaved in the same way. The hydrogen sulphates of 2-amino-1-naphthol and of 2-amino-6-naphthol and their corresponding acetamido derivatives were separated by descending development in solvent system (*d*) on no. 3MM paper (for R_F values see Booth *et al.* 1955). By development in this solvent system and acid treatment of the chromatogram at 70°, 2-acetamido-6-naphthyl hydrogen sulphate was identified in the urine of both species but 2-acetamido-1-naphthyl hydrogen sulphate was not found in either species. The detection of 2-amino-1-naphthol after hydrolysis of the synthetic hydrogen sulphate was carried out by exposing the spot to ammonia vapour, when a characteristic blue colour was obtained.

Both rat and rabbit urine contained a compound believed to be 2-acetamido-5:6-dihydro-5:6-dihydroxynaphthalene. The occurrence of this compound as a glucosiduronic acid and some reasons for the structure suggested have been described (Boyland & Manson, 1957). The unconjugated compound was not readily extracted by ether even by continuous extraction but was extracted by ethyl acetate or butanol. The compound was not detected in the urine of rabbits dosed with 2-amino-6-naphthol, only 2-amino-6-naphthol and 2-acetamido-6-naphthol and their hydrogen sulphates and glucosiduronic acids being found.

Neither 2-amino-1-naphthol nor 2-acetamido-1-naphthol was detected in the urine of rats or rabbits by paper chromatography. The injection of 2-acetamidonaphthalene into rabbits gave the same metabolites in the urine as injection of 2-naphthylamine, except that no 2-naphthylamine was detected. The injection of 2-acetamidonaphthalene into rats gave the same compound as injection of the parent amine except that 2-amino-6-naphthol and its hydrogen sulphate were not found.

Chemical oxidation of 2-acetamidonaphthalene

2-Acetamidonaphthalene (0·9 g.) was oxidized by the chemical hydroxylating system (ascorbic acid, ferrous sulphate, ethylenediaminetetra-acetic acid and oxygen) of Udenfriend, Clark, Axelrod & Brodie (1954) and Brodie, Axelrod, Shore & Udenfriend (1954), as employed by Booth *et al.* (1955). The reaction mixture was extracted several times with ether and then with ethyl acetate. The ethereal extracts contained the compounds giving the reactions and R_F values [0·95 and 0·92 in solvent system (*a*)] of 2-acetamido-1-naphthol and 2-acetamido-6-napthol (Booth *et al.* 1955). The ethyl acetate extracts were evaporated to dryness and examined by paper chromatography. They contained a compound [R_F 0·8 in solvent system (*a*)] with identical properties to those of the metabolite described in this paper and by Boyland & Manson (1957), which is believed to be 2-acetamido-5:6-dihydro-5:6-dihydroxynaphthalene. A two-dimensional ascending chromatogram was carried out, developing first in solvent system (*a*), then treating with acid at 70°, drying and developing in the second direction with solvent system (*b*). A spot [R_F 0·8 in solvent system (*b*)] with the colour reactions of 2-amino-6-naphthol was detected.

DISCUSSION

2-Acetamido-6-hydroxy-5-naphthyl hydrogen sulphate is a new metabolite of 2-naphthylamine and 2-acetamidonaphthalene. Boyland & Sims (1957) isolated an analogous compound, 2-hydroxy-1-naphthyl hydrogen sulphate, from rabbits dosed with naphthalene. Other workers (Corner & Young, 1954, 1955; Sato, Yamada, Suzuki, Fukuyama & Yoshikawa, 1956; Solomon, 1954) detected this conjugate in the urines of several species after the injection of naphthalene. Dobriner *et al.* (1941) stated that they had isolated an unknown metabolite from the urine of rats injected with 2-naphthylamine and that its properties suggested that it was a dihydroxyaminonaphthalene but it was not further characterized. The urine of rabbits and monkeys contained only traces of this compound.

Evidence for the presence of 2-acetamido-5:6-dihydro-5:6-dihydroxynaphthalene is confined to the behaviour of the suspected metabolite on paper chromatograms (this paper and Boyland & Manson, 1957), but the presence of this compound would be expected by analogy with the metabolism of naphthalene (Young, 1947; Booth & Boyland, 1949). Moreover, in the metabolism of naphthalene, 1:2-dihydro-1:2-dihydroxynapthalene appears to be an intermediate in the formation of 1:2-dihydroxynaphthalene (Corner & Young, 1955; Sato *et al.* 1956). 2-Naphthylamine and 2-acetamidonaphthalene are metabolized to similar derivatives. Of the metabolites detected by paper chromatography, 2-amino-1-naphthyl hydrogen sulphate has been isolated from the urine of various species after administration of 2-naphthylamine (Boyland *et al.* 1957; Manson & Young, 1950; Wiley, 1938) and 2-acetamido-6-naphthol has been isolated from the urine of animals injected with 2-naphthylamine and 2-acetamidonaphthalene (Dobriner *et al.* 1941; Manson & Young, 1950). The latter may be an artifact formed by enzymic hydrolysis of conjugates in the urine. 2-Acetamidonaphthalene is also a known metabolite of 2-naphthylamine (Dobriner *et al.* 1941). The identical properties of 2-diacetamidonaphthalene and 2-acetamidonaphthalene in the solvent systems used do not allow the former compound to be excluded as a metabolite. Peters & Gutmann (1955) found that 2-diacetamidofluorene was a metabolite of 2-aminofluorene in rat-liver slices.

2-Acetamido-1-naphthyl hydrogen sulphate was not detected in the urine of either species. Booth *et al.* (1955) found that this compound was deacetylated by rat-kidney and -liver slices and also that 2-amino-1-naphthyl hydrogen sulphate was not acetylated by these tissues. Hence the excretion of 2-acetamido-1-naphthyl hydrogen sulphate is unlikely and in fact only 2-amino-1-naphthyl hydrogen sulphate was detected. On the other hand, 2-acetamido-6-naphthyl hydrogen sulphate was not deacetylated by rat-liver and -kidney slices and 2-amino-6-naphthyl hydrogen sulphate was acetylated. 2-Acetamido-6-naphthyl hydrogen sulphate was detected in the urine of both species. The metabolites of 2-naphthylamine are listed in Table 1.

SUMMARY

1. The isolation of 2-acetamido-6-hydroxy-5-naphthyl hydrogen sulphate from the urine of rabbits injected with 2-acetamidonaphthalene is described. The compound was detected in the urine of rabbits and rats injected with 2-naphthylamine.

2. The compound was synthesized by the persulphate oxidation of 2-nitro-6-naphthol followed by reduction and acetylation with thioacetic acid.

3. The urine of rats and rabbits injected with 2-naphthylamine was examined by paper chromatography. 2-Naphthylamine, 2-acetamidonaphthalene, 2-acetamido-6-naphthol, 2-amino-1-naphthyl hydrogen sulphate, 2-acetamido-6-naphthyl hydrogen sulphate and a compound which is probably 2-acetamido-5:6-dihydro-5:6-dihydroxynaphthalene were detected. Rat urine sometimes contained 2-amino-6-naphthol and its hydrogen sulphate. The injection of 2-acetamidonaphthalene gave the same products except that 2-naphthylamine was not detected in rabbit urine and 2-amino-6-naphthol and its hydrogen sulphate were not detected in rat urine. 2-Amino-1-naphthol, 2-acetamido-1-naphthol and 2-acetamido-1-naphthyl hydrogen sulphate were not detected as metabolites of 2-naphthylamine or 2-acetamidonaphthalene in the urine of rats or rabbits.

4. The oxidation of 2-acetamidonaphthalene by an ascorbic acid–ferrous sulphate–oxygen system yields a compound which is probably 2-acetamido-5:6-dihydro-5:6-dihydroxynaphthalene.

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The Amino Acid Composition of Cytochrome *c* from Horse Heart

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Though the physiological properties of haemoproteins such as haemoglobin, catalase, peroxidase and cytochrome *c* ultimately depend on the structure of their common prosthetic group they differ so widely that the properties of the prosthetic group must be greatly affected by the nature of the protein to which it is attached and the way in which it is bound to the protein. Our understanding of this problem is limited largely by our ignorance of the structure of the proteins involved. A certain amount is known about the relation between the protein and haem parts of the molecule of cytochrome *c*. Theorell (1938, 1939) discovered that the prosthetic group is firmly attached to the protein by thioether linkages with two cysteine residues. Tsou (1951) subsequently isolated from a peptic hydrolysate of cytochrome *c* a peptide carrying the prosthetic group, and the amino acid sequence of this peptide has been worked out (Tuppy & Paléus, 1955; Leaf & Gillies, 1955). Tuppy & Bodo (1954) also examined similar peptides obtained by tryptic and acid hydrolysis. As none of these peptides possesses the specific enzymic properties of cytochrome *c*, however, the remainder of the peptide chain must contribute significantly to the properties of the intact protein.

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