Enzymic and Chemical Properties of Cytoplasmic Particles from Wheat Roots

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Studies of the enzymic and chemical properties of microsomes and mitochondria isolated by differential centrifuging of dispersions of white petioles of silver beet (*Beta vulgaris*) have been reported previously (Martin & Morton, 1956*a, b*). An active cytochrome *c* reductase system, with reduced di- (and tri-)phosphopyridine nucleotides (DPNH and TPNH) as substrates, was found to be associated with the beet microsomes. This system was unaffected by the antibiotic antimycin A. The microsomes showed negligible succinic dehydrogenase activity. In contrast, the mitochondria had an antimycin A-sensitive succinic dehydrogenase system, as well as a DPNH- (and TPNH-)cytochrome *c* reductase system which was partially inhibited by antimycin A. The microsomes contained slightly more ribonucleic acid phosphorus (RNA-P) than the mitochondria, and about three times the lipid phosphorus of these particles.

The white petiole of silver beet was chosen for these studies largely on the basis of enzymic activities and the availability of the material (Martin & Morton, 1956*a*). However, this is a relatively dormant plant tissue, essentially structural in nature. To extend the investigation to a distinctly different and rapidly growing tissue, similar studies have now been carried out on the roots of germinating wheat. The investigation has been facilitated by the ready availability of germinating wheat from a commercial source. The results obtained with the two plant materials are compared in this paper. This work was briefly described at the thirty-first meeting of the Australian and New Zealand Association for the Advancement of Science, Melbourne, in August 1955.

MATERIALS AND METHODS

Except where otherwise indicated, the sources of chemicals and the enzymic and chemical methods used in this study were essentially the same as described in the previous papers (Martin & Morton, 1956*a, b*).

Wheat roots. The wheat used had been germinating for some 48 hr. under conditions used for commercial malting. The developing roots were about 5–10 mm. long. They were removed by vigorous rubbing of the grain by hand on a stainless-steel wire screen (no. 8 mesh, with apertures 2.5 mm. $\times$ 2.5 mm.). The material which fell through the screen con-

sisted mostly of roots together with some coleoptiles and broken grains. The yield was about 20 g./kg. of wheat.

Dispersion medium. The simple medium used for isolation of beet-petiole particles (Martin & Morton, 1956*a*) was somewhat modified. The medium for dispersion of the wheat roots consisted of 0.2M sucrose, 0.1M potassium phosphate buffer (pH 7.4), 0.1M potassium succinate, 0.01% (w/v) ethylenediaminetetraacetate (EDTA, used as the disodium salt Versene) and 0.1% (w/v) cysteine hydrochloride. The last-named was added to the medium immediately before use. The interference of the potassium succinate, EDTA and the cysteine hydrochloride with the enzymic studies was eliminated by washing the sedimented particles as described below. These compounds were included in the dispersion medium to inhibit the polyphenol oxidase activity, and to protect the succinic dehydrogenase and cytochromes by keeping them reduced (see Keilin & Hartree, 1940; Morton, 1955*a*).

EXPERIMENTAL AND RESULTS

Isolation of cytoplasmic particles

All operations were carried out in a cold room at about 2–4°. Approx. 100 g. of roots was ground by hand with 25 g. of washed sand and 200 ml. of dispersion medium, in a large pre-chilled earthenware mortar and pestle. The material was then filtered through four thicknesses of cheese cloth. For each preparation, usually 400 g. of roots was so treated, yielding about 700 ml. of filtered dispersion. The fractionation was carried out at 0–4°, with slight modifications of the procedure previously described (Martin & Morton, 1956*a*). Fig. 1 shows the procedures. An International refrigerated centrifuge, a Servall model SS2 centrifuge, and a Spinco Model L centrifuge were used successively for sedimentation of the 1500 g, 15 min. fraction, the mitochondria and the microsomes respectively. In each case the centrifuge head was pre-cooled to 0°.

To reduce the time taken for the isolation of the microsome fraction for the enzymic studies, the separation of a fraction at 20000 g for 15 min. was omitted, but this was removed during isolation of the microsome fraction for the chemical analyses, as shown in Fig. 1. The mitochondria and microsomes for the enzymic studies were finally suspended in 0.2M potassium phosphate buffer (pH 7.4) at 0° and oxygen was passed through the suspension for about 5 min. to remove any traces of cysteine remaining from the dispersion medium. For the chemical analyses, the washed particles were dried over P_2O_5 in *vacuo* at room temperature. The yields of washed particles from 100 g. of wheat roots were about 170 mg. of mitochondria and 80 mg. of microsomes,

compared with about 20 and 10 mg. respectively, obtained from 100 g. of beet petioles by the procedure of Martin & Morton (1956a).

Enzymic activities of cytoplasmic particles from wheat roots

Table 1 gives the succinate-cytochrome *c* reductase and DPNH-cytochrome *c* reductase activities of mitochondria and microsomes from

wheat roots. The effects of antimycin A on these activities is also shown. Although the mitochondria have an active reductase system with sodium succinate as substrate, the microsomes have negligible activity. The DPNH-cytochrome *c* reductase activity of the mitochondria is partially inhibited by antimycin A at high concentrations, and the similar activity of the microsomes is not inhibited.

Fig. 1. Scheme for isolation of cytoplasmic particles from dispersions of wheat roots.

Table 1. *Effect of antimycin A on the succinate- and DPNH-cytochrome c reductase activities of mitochondria and microsomes from wheat roots*

Mitochondria and microsomes were isolated from dispersions of roots from germinating wheat by differential centrifuging. The rate of enzymic reduction of heart-muscle cytochrome *c* at about 20° was measured spectrophotometrically as previously described (Martin & Morton, 1956*a*). The final concentrations of reactants in the cuvette were: 0.066*M* sodium-potassium phosphate buffer (pH 7.4); 0.001*M*-KCN; 10⁻⁴*M* oxidized cytochrome *c*; 10⁻⁴*M* DPNH, or 0.06*M* sodium succinate. The reaction was commenced by addition of the substrate. Results are expressed as μ moles of cytochrome *c* reduced/hr./mg. of N.

Cytoplasmic fraction	Substrate	Antimycin A concn.		Enzymic activities		Inhibition (%)
		μ g./mg. of particle N	μ M	Control	With antimycin A	
10000 g, 15 min. (mitochondria)	Succinate	0.15	0.006	11.3*	6.8*	40
	DPNH	0.77	0.006	52.2	50.2	4
		3.9	0.03	51.6	42.1	18
		77	0.60	63.5	46.2	27
		390	3.0	58.4	40.8	30
60000 g, 60 min. (microsomes)	Succinate	—	—	0	—	—
	DPNH	—	—	145	—	—
		830	3.0	150	148	0

* Tested immediately after isolation of the particles.

Table 2. *Effect of storage time on the inhibition of succinate-cytochrome c reductase activity of mitochondria from wheat roots*

Activities were determined at various times after isolation of the mitochondrial fraction. Storage was at room temp. (approx. 20°). Activities were determined as for Table 1 and are expressed as μ moles of cytochrome *c* reduced/hr./mg. of N.

Expt.	Storage time (min.)	Antimycin A concn.		Potassium malonate concn. (μ M)	Enzymic activities		Inhibition (%)
		μ g./mg. of mitochondrial N	μ M		Control	With inhibitor	
A	0	0.15	0.006	0	11.3	6.8	40
	0	15	0.60	0	10.9	5.7	48
	120	75	3.0	0	5.7	0.09	98
	130	15	0.60	0	4.4	0.03	99
B	0	37	0.60	0	11.0	5.5	50
	0	0	0	66	11.0	0	100
	90	37	0.60	0	5.4	0.3	94
	100	0	0	66	5.1	0	100

As shown in Table 2, the succinate-cytochrome *c* reductase activity of wheat-root mitochondria, isolated as described above, declines rapidly if the suspension is stored at room temperature (about 20°). Not only does the overall level of activity decline, but so does the residual activity remaining after treatment with antimycin A. The percentage inhibition by antimycin A therefore varies according to the period elapsed between isolation and testing of the mitochondria. However, the inhibition by potassium malonate (66 μ M) is virtually complete at any period of storage (Table 2).

Chemical composition of cytoplasmic particles from wheat roots

Microsomes. Lipid, RNA, deoxyribonucleic acid (DNA) and protein were estimated by procedures essentially similar to those used for analyses of

particles from beet petiole (Martin & Morton, 1956*b*). RNA was extracted with *N*-HClO₄ at 2–4° for 30 hr. and so-called DNA with 10% (w/v) KCl (buffered at pH 4.0) for 20 min. at about 100°. The results are shown in Table 3.

The base compositions of the HClO₄ and KCl extracts were determined by paper chromatography as already described (Martin & Morton 1956*b*). The relative amounts of adenine, guanine, cytosine and uracil in the HClO₄ extract were essentially as found in the similar extract of petiole microsomes (Martin & Morton, 1956*b*). No thymine was detected in this extract, which contained 25 moles of uracil/100 g. atoms of applied P, about 88% of the applied nucleic acid phosphorus being recovered from the chromatogram.

Both uracil and thymine were detected in the hydrolysate of the KCl extract. By assuming that

wheat-root DNA contains 27 moles of thymine/100 g. atoms of P, as does wheat-germ DNA (Chargaff, 1955; see Martin & Morton, 1956*b*), it was calculated from the proportion of bases that the salt extract contained mainly RNA (85%) with some DNA (10% of the total nucleic acid). About 5% of the applied nucleic acid phosphorus was not recovered from the chromatogram. Estimation of the deoxyribose in the KCl extract indicated that about 13% of the total nucleic acid phosphorus was DNA-P (see Table 3). The higher estimate from the colorimetric determination of sugar, as compared with that from estimation of thymine, is in general agreement with the previous findings with microsomes from beet petioles (Martin & Morton, 1956*b*).

Mitochondria. For the analysis of mitochondria, the treatment with $N-HClO_4$ was omitted and the total nucleic acid phosphorus extracted with buffered 10% (w/v) KCl at about 100° for 20 min. The results are shown in Table 3. The base composition of the mitochondrial extract was not investigated.

DISCUSSION

Cytoplasmic particles from wheat roots

In contrast to the white petiole of silver beet, which contains mostly mature, well-vacuolated cortical cells and fibres, the wheat roots used in the present

study contain mostly small, non-vacuolated meristematic cells. The much greater amount of cytoplasmic material in the wheat-root cells is reflected by the higher yields of cytoplasmic particles from these as compared with the yields from beet petioles. Electron micrographs (Hodge, Martin & Morton, unpublished work) show that the wheat-root microsomes consist mainly of vesicular structures studded with electron-dense granules and that they originate from the cellular endoplasmic reticulum.

Enzymic activities of wheat-root particles

The results shown in Table 1 are in agreement with the distribution of enzymic activities among mitochondria and microsomes as found previously with beet petiole (Martin & Morton, 1956*a*). The DPNH-cytochrome *c* reductase activity of the microsomes is about three times that of the reductase activity of the mitochondria and the two activities are differentiated by their response to the antibiotic antimycin A. The general level of DPNH-cytochrome *c* reductase activity of the wheat-root particles is much higher than that of petiole particles (mitochondria about 60, as compared with 14; microsomes about 150, as compared with about 70 μ moles of cytochrome *c* reduced/hr./mg. of N). This difference probably reflects the greater synthetic activity of the wheat roots. The pro-

Table 3. *Chemical composition of cytoplasmic particles from wheat roots*

The analyses were carried out as described in the text. Results for mitochondria are the means of duplicates, and for microsomes the means of triplicates. After removal of lipids, 'acid-soluble phosphates' were extracted with 0.2 $N-HClO_4$ at 2-4° but the amounts were not estimated. 'Total RNA-P' for microsomes is the sum of RNA-P extracted with cold $N-HClO_4$ at 0° and that extracted by hot KCl (10%, w/v); for mitochondria it is the difference of the DNA-P estimate (by determination of deoxyribose) from the total nucleic acid P (NA-P). Deoxyribose was estimated by the Ceriotti (1952) procedure. Results are expressed as % dry wt.

Extraction procedure	Determination	Mitochondria		Microsomes Prep. I
		Prep. I	Prep. II	
Methanol, $CHCl_3$ -methanol, <i>n</i> -butanol and ether at room temp.	Total lipid	—	—	46.5
	Lipid P	0.120	0.094	0.066
	P as % of total lipid	—	—	0.142
$N-HClO_4$ at 2-4°, 30 hr.	RNA-P	—	—	—
	Direct	—	—	0.116
	From absorption	—	—	0.111
10% (w/v) KCl at 100°, 20 min.	NA-P	—	—	—
	Direct	0.143	0.170	0.089
	From absorption	0.075	0.086	0.079
	DNA-P from deoxyribose	0.021	0.027	0.011
	RNA-P*	0.054	0.059	0.068
$N-KOH$ at 100° for 15 min., then $N-H_2SO_4$, 100° for 15 min.	Protein	—	—	—
	Nitrogen	5.87	6.20	4.65
	Protein ($N \times 6.25$)	36.7	38.8	29.1
	Total RNA-P	0.054	0.059	0.179
	Total NA-P†	0.075	0.086	0.190
	μ g. of RNA-P/mg. of protein N‡	9.2	9.5	38.7
	μ g. of DNA-P/mg. of protein N‡	3.6	4.4	2.4

* Difference between NA-P (from absorption) and DNA-P (from deoxyribose value).

† Sum of 'Total RNA-P' and 'DNA-P from deoxyribose'.

‡ Based on values for 'Total RNA-P' and 'DNA-P from deoxyribose'.

tective action of the dispersion medium used in isolation of the root particles may also partly contribute to it. The DPNH-cytochrome *c* reductase activity of wheat-root microsomes appears to be intimately associated with cytochrome b_5 (Martin & Morton, 1957) as is that of beet microsomes (Martin & Morton, 1955).

*Succinate-cytochrome *c* reductase activity.* The succinic dehydrogenase system of wheat-root mitochondria shows some unusual properties as compared with that of beet-petiole mitochondria. When freshly isolated from petioles, the beet mitochondria have a reductase activity (11.2 μ moles of cytochrome *c* reduced/hr./mg. of N; Martin & Morton, 1956*a*; Table 3) similar to that of freshly isolated wheat-root mitochondria (11.3 μ moles of cytochrome *c* reduced/hr./mg. of N; Table 2). However, the activity of the beet mitochondria is strongly (93%) inhibited by low levels of antimycin A (0.06 μ M; 5 μ g./mg. of mitochondrial N). In contrast, when freshly isolated only 40–50% of the activity of the wheat-root mitochondria is inhibited by antimycin A (0.60 μ M; 15 μ g./mg. of mitochondrial N) (Table 2). After storage, the same concentration of antimycin A inhibits the activity almost completely. Table 2 shows that the activity lost during storage (5.6 units between 0 and 120 min.) is almost the same as the activity resistant to 0.60 μ M antimycin A immediately after isolation (5.2 units). The complete inhibition by potassium malonate (66 μ M), both initially and after 100 min. of storage, indicates that the activities are associated with an enzyme (or enzymes) highly specific towards the substrate.

The results suggest that wheat-root particles have a substantial reductase activity which is resistant to antimycin A but is very labile on storage in an oxygenated environment. Because of the storage lability of this antimycin A-resistant activity, it may not be detected in particles sedimented in the usual way from 0.25M sucrose according to the widely used procedure of Schneider & Hogeboom (1950). Therefore, although not found in mitochondria isolated from beet petioles, the possible existence of this pathway in such mitochondria cannot be excluded.

As in animal tissues (Morton, 1955*b*), so in plant tissues the succinic dehydrogenase and fumaric hydrogenase activities are associated with the one enzyme (Martin & Morton, 1956*a*). This is almost certainly a flavoprotein resembling the fumaric hydrogenase of yeast (see Morton, 1955*b*). Cytochrome *dh* (cytochrome 'dehydrogenase') described by Lundegårdh (1954) and considered by him to be identical with succinic dehydrogenase in wheat roots, could not be identified in the wheat-root mitochondrial preparations (Martin & Morton, 1957). However, the true nature of the plant-

succinic dehydrogenase system, and the possible existence of two distinct malonate-sensitive dehydrogenases in wheat roots, can only be established finally by purification studies. It is recalled that mitochondria from plants (Martin & Morton, 1956*a*, and Table 2), as well as from animals (Duve, Pressman, Gianetto, Wattiaux & Appelmans, 1955) have a DPNH-cytochrome *c* reductase which, like wheat-root succinate-cytochrome *c* reductase, is only partially inhibited by high concentrations of antimycin A. The recent observations of Ernster, Jalling, Löw & Lindberg (1955) strongly suggest that this partial inhibition is due to the existence of two distinct mitochondrial pathways for oxidation of DPNH, only one of which is sensitive to antimycin A.

Chemical composition of wheat-root particles

The analytical methods used were substantially those developed for analysis of particles from silver-beet petioles (Martin & Morton, 1956*b*). Good agreement between values for nucleic acid phosphorus, estimated directly as organic P and calculated from the value of $\lambda_{\max}$, determined spectrophotometrically, established the validity of the separation of lipid, nucleic acid and protein P of the beet particles (see Martin & Morton, 1956*b*, Table 7). Table 3 shows that the same good agreement between the two estimates was not obtained in the analyses of the wheat-root particles. The discrepancy is slight with microsomes, but considerable for the mitochondrial fraction. Since the ultra-violet absorption curves of the extracts of both the mitochondria and microsomes agreed well with those expected for plant nucleic acid (see Martin & Morton, 1956*b*, Fig. 1), the figures based on the values at $\lambda_{\max}$ are preferred as the true estimates of nucleic acid phosphorus. These figures have, therefore, been used in calculating the quotient μ g. of RNA-P/mg. of protein, shown in Table 3.

The discrepancy between the two estimates of nucleic acid phosphorus in the potassium chloride extract of mitochondria probably is due to the incomplete extraction of some organo-phosphorus compound by the lipid solvents. The sum of the lipid P and organic P in the potassium chloride extract is almost the same for preparations I and II (0.264 and 0.263% dry wt. respectively; see Table 3), but the distribution of this total P differs. This emphasizes the necessity of confirming analytical results obtained for plant tissues by as many procedures as possible (see Martin & Morton, 1956*b*).

With reservations arising from the known lack of homogeneity of the wheat-root preparations, the results for the wheat-root particles (Table 3) may be compared with those previously reported for beet-petiole mitochondria and microsomes (Martin & Morton, 1956*b*, Tables 4, 7 and 9).

Microsomes from both sources contain a very high proportion of lipid (wheat root, 46.5; beet petiole, 48.6–56.9% dry wt.). Lipid and protein together comprise the greater part (75–90%) of the dry wt. of these particles from both sources. However, the lipid P (0.066% dry wt.) is markedly less for the wheat-root particles than for those from beet petiole (0.687–0.760% dry wt.).

Although mitochondria from the two sources have similar proportions of RNA-P (wheat roots, 9.2 and 9.5; beet petioles, 6.8–8.0 μ g. of RNA-P/mg. of protein N), microsomes from wheat roots contain much more RNA-P than do those from silver beet (0.179, as compared with 0.053–0.076% dry wt.; or 38.7, as compared with 9.6–24.0 μ g. of RNA-P/mg. of protein N). However, the proportion of DNA-P in the microsomes is similar (wheat roots, 2.4; beet petioles, 1.3–2.7 μ g. of DNA-P/mg. of protein N). As indicated previously (Martin & Morton, 1956b), the higher RNA content of wheat-root microsomes reflects the greater number of small granules (about 10 m μ .) associated with the microsome fraction. This and other information indicates that most of the RNA of plant microsomes is associated with such granules (see Martin & Morton, 1956b).

Like microsomes from silver beet, those from wheat roots contain two types of RNA (above and Table 3). One type is extracted with cold n -perchloric acid, and the other is extracted on further treatment with hot potassium or sodium chloride (10%, w/v). It has been suggested previously (Martin & Morton, 1956b) that this latter form of RNA may be associated with the lipoprotein matrix of the structural elements of the endoplasmic reticulum, and the more easily extractable RNA is associated with the RNA-rich granules.

Correlation between plant-protein synthesis and microsomal RNA

The rate of protein synthesis by the rapidly growing roots of germinating wheat very greatly exceeds that of the comparatively dormant beet petiole. As indicated above, the RNA content of wheat-root microsomes is much higher than that of beet-petiole microsomes, and the RNA content of the mitochondria from the two sources differs very little. This argues against the direct involvement of mitochondrial RNA in protein synthesis in plant cells. The RNA of plant mitochondria is possibly associated with the oxidative phosphorylation carried out by these granules. By contrast, the increased number of RNA-rich granules associated with the wheat-root microsomes strongly suggests that this RNA is directly involved in protein synthesis in the cytoplasm of plant cells. The RNA-rich granules probably function in the uptake and

incorporation of amino acids during the primary steps of protein synthesis, as do the RNA-rich granules of liver (Littlefield, Keller, Gross & Zamecnik, 1955). The microsomal system for oxidation of DPNH (Bailie & Morton, 1955; Martin & Morton, 1955, 1956a, b) may provide energy for these primary steps.

SUMMARY

1. Roots were separated from wheat which had been germinated for 48 hr. under commercial conditions of malting. Cytoplasmic particles were separated from the dispersion of disintegrated roots by differential centrifuging. Only the so-called 'mitochondrial' and 'microsomal' fractions were studied.

2. Whereas the mitochondria showed a high succinate-cytochrome *c* reductase activity, none was detected in the microsome fraction. The mitochondrial reduced diphosphopyridine nucleotide-cytochrome *c* reductase activity was partially (30%) inhibited by antimycin A (3 μ M, 370 μ g./mg. of mitochondrial nitrogen), but the similar activity of the microsomes was not inhibited by antimycin A (3 μ M, 830 μ g./mg. of microsomal nitrogen).

3. The wheat-root mitochondrial preparation appears to have two distinct malonate-sensitive pathways for coupling of oxidation of sodium succinate to reduction of cytochrome *c*. One pathway is not inhibited by antimycin A, but is particularly labile on storage under oxidative conditions; the other is strongly inhibited by low concentrations of antimycin A, and somewhat less labile on storage. The latter corresponds to the well-known succinic dehydrogenase system of animal tissues.

4. Analyses of lipid, nucleic acid and protein of wheat-root mitochondria and microsomes are presented. Compared with the similar particles from silver-beet petiole, wheat-root microsomes are very rich in ribonucleic acid, and the mitochondria from both sources are substantially similar in this respect. Wheat-root microsomes, like those from silver-beet petiole, contain two types of ribonucleic acid as determined by relative resistance to extraction procedures.

5. Comparison of the results for the rapidly growing wheat roots with those for the relatively dormant beet petioles indicate that the ribonucleic acid content of the microsomes, but not of the mitochondria, is correlated with protein synthesis.

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Uridine Diphosphate Glucuronic Acid as Glucuronyl Donor in the Synthesis of 'Ester', Aliphatic and Steroid Glucuronides

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There are two types of glucuronide, depending on the aglycone: alcohols and phenols give rise to glycosidic or 'ether'-linked glucuronides, and certain carboxylic acids to acylal or 'ester'-linked conjugates (see Teague, 1954).

It is known that uridine diphosphate glucuronic acid (UDPGlucuronic acid) acts as a glucuronyl donor in the synthesis of 'ether' glucuronides, transferring its glucuronic acid to an acceptor (R.OH), an 'ether' glucuronide and uridine 5'-pyrophosphate (UDP) being formed in the following manner (Dutton & Storey, 1953, 1954; Storey & Dutton, 1955; Isselbacher & Axelrod, 1955):

Storey & Dutton (1955) suggested that UDP-glucuronic acid is an 'active' form of glucuronic acid, taking part in the general tissue metabolism of that substance; it therefore seemed of interest to study the range of compounds acting as acceptors in the above reaction. The aglycones previously known to be conjugated in this manner were o-aminophenol, (-)-menthol (Dutton & Storey, 1951), phenolphthalein, thyroxine and some corticosteroids (Isselbacher & Axelrod, 1955), all yielding 'ether' glucuronides.

The present work extends these observations to include as acceptors two aromatic acids forming 'ester' glucuronides and, as examples of aliphatic compounds, an acid and an alcohol giving rise to an 'ester' and an 'ether' type of conjugate re-

spectively. Evidence for the formation of the glucuronides of pregnane-3 α :20 α -diol and of some other compounds is also presented.

Of the aglycones chosen, *p*-aminobenzoic acid has been isolated as its 'ester' glucuronide from rabbit urine (Bray, Lake, Neale, Thorpe & Wood, 1948); formation of *o*-aminobenzoyl glucuronide has been reported in rat-liver slices (Shirai & Ohkubo, 1954a); 1:1-dimethylpropanol (*tert*-amyl alcohol) and α -ethylhexanoic acid are excreted as 'ether' and 'ester' glucuronides respectively in rabbit urine (Kamil, Smith & Williams, 1953a, b). Pregnane-3 α :20 α -diol and 3 α -hydroxyandrostane-17-one (androsterone) glucuronides have been isolated from human urine (Venning & Browne, 1936; Edwards & Kellie, 1956), and 4:4'-dihydroxy- $\alpha\beta$ -diethylstilbene (stilboestrol) glucuronide from rabbit urine (Mazur & Shorr, 1942); *allopregnan*-3 β :20 β -diol glucuronide does not yet appear to have been detected, although the 3 α :20 α -isomer has been found in human urine, conjugated with an unidentified substance (Kyle & Marrian, 1951).

Preliminary reports of this work have already appeared (Dutton, 1955; Storey & Dutton, 1956; Dutton & Spencer, 1956).

EXPERIMENTAL

Materials

Acceptor substrates and reference compounds. *o*-Aminobenzoic (OAB) and *p*-aminobenzoic (PAB) acids were commercial samples thrice recrystallized from water.