formation of 3,5-dihydroxyphenylacetic acid, the former was incubated under similar conditions with intestinal micro-organisms, when the formation of 3,5-dihydroxyphenylacetic acid was shown. Administration of 3,4,5-trihydroxyphenylacetic acid to rats resulted in the excretion of 3,5-dihydroxyphenylacetic acid.

To determine whether myricetin breakdown is dependent on the activity of the intestinal microflora *in vivo*, the antibiotic neomycin (100mg) was fed daily to rats before and during the period of myricetin administration, when the formation of 3,5-dihydroxyphenylacetic acid was suppressed.

Incubation of the glycoside myricitrin (myricetin 3-rhamnoside) with intestinal micro-organisms gave rise to free myricetin, 3,4,5-trihydroxyphenylacetic acid and 3,5-dihydroxyphenylacetic acid, indicating that rat intestinal micro-organisms are capable of effecting the cleavage of the glycosidic linkage as well as the heterocyclic ring system of myricitrin.

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The Metabolic Fate of 4-Chloro-2-methylphenoxyacetic Acid in Peas

By D. J. COLLINS and J. K. GAUNT. (*Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Caerns., U.K.*)

This report describes preliminary results from an investigation into the metabolism of the selective herbicide, MCPA,* in plants.

Radioactive MCPA, ^{36}Cl- or *carboxy*-^{14}C-labelled, was applied to the leaves of 21-day-old pea plants (*Pisum sativum* var. Progress no. 9). After 24h the plants were extracted with boiling ethanol. Extracts were concentrated and fractionated by partition into ether. Fractions were examined by t.l.c. for labelled metabolites. Two of the breakdown products (MX_1 and MX_2) were found to be ether-soluble, whereas a third was not. In each case both the chlorine atom and the carboxyl carbon remained on the molecule.

This application technique did not permit the use of enough herbicide to isolate sufficient of any metabolite for its chemical identification. Thus incubation experiments with excised plant tissue were set up. Light-grown pea shoot sections were incubated for 48h in a medium containing 0.5mM-MCPA. Examination of tissue extracts showed the presence of all three previously observed metabolites. Repeated t.l.c. of ether-soluble fractions led to the isolation of products MX_1 and MX_2 as crystalline compounds.

Both substances are non-phenolic acids with u.v. spectra identical with that of MCPA. Compound MX_1 has an i.r. spectrum characteristic of a hydroxylated compound. It was shown to be 4-chloro-2-hydroxymethylphenoxyacetic acid, after synthesis of this compound. Compound MX_2 has an i.r. spectrum characteristic of an amido acid. Acid and alkaline hydrolysis yielded MCPA and a ninhydrin-positive ether-insoluble component. Though still impure, the chemical, chromatographic and spectroscopic properties of compound MX_2 are similar to those of synthetic *N*-(4-chloro-2-methylphenoxyacetyl)-L-aspartic acid.

The ether-insoluble metabolite is also acidic and unstable to hydrolysis. Its purification and chemical characterization are currently under investigation.

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* Abbreviation: MCPA, 4-chloro-2-methylphenoxyacetic acid.

The Microbial Metabolism of Biphenyl

By D. LUNT and W. C. EVANS. (*Department of Biochemistry and Soil Science, University College of North Wales, Bangor, Caerns., U.K.*)

Pure cultures of Gram-negative bacteria were isolated from tropical and temperate soils capable of utilizing biphenyl as sole carbon source in an otherwise mineral salts medium. 2,3-Dihydroxybiphenyl was detected and isolated from the culture; this was cleaved by a particulate fraction from biphenyl-grown cells to give α-hydroxy-β-phenylmuconic semialdehyde [(m.p. 122°C, $\lambda_{max.}$ 350nm (acid) and 430nm (alkali)]. A soluble cell-free extract converted this initial ring-fission product into phenylpyruvate through some, as yet, unidentified intermediates. The subsequent metabolic pathways of phenylpyruvate are known. Unsubstituted biphenyl is therefore hydroxylated in the 2,3-positions, to be followed by a '*meta*'-type cleavage between C-3 and C-4 of the aromatic ring; this is to be contrasted with the fate of naphthalene, where a '*meta*'-type fission occurs between the angular carbon atom and C-1 of 1,2-dihydroxynaphthalene (Davies & Evans, 1964). Several plant aromatic polymers contain the biphenyl type bond (e.g. the biflavanoids).

Multicomponent mixtures of chlorinated bi-

phenyls are used in the plastics industry, and their persistence in the environment is giving cause for concern; it may be possible to select those with the desired properties and still be biodegradable.

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Cinnamic Acid and *p*-Coumaric Acid, Precursors of Ubiquinone in Higher Plants, Green Algae and Fungi

By D. R. THRELFALL, AH LAW and G. R. WHISTANCE. (*Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth, U.K.*)

Previously we have reported on the incorporation of *p*-hydroxybenzoic acid into ubiquinone by the higher plants maize and French bean, the green algae *Euglena gracilis* and *Ochromonas danica*, the moulds *Agaricus campestris* and *Phycomyces blakesleeanus*, and the yeast *Saccharomyces cerivisiae* (Whistance, Threlfall & Goodwin, 1967; Spillor, Threlfall & Whistance, 1968; Threlfall & Whistance, 1970). We now report on the incorporation into ubiquinone by maize, *E. gracilis*, *P. blakesleeanus* and baker's yeast of cinnamic acid and *p*-coumaric acid, compounds known to give rise to p-hydroxybenzoic acid in higher plants (Zenk, 1966) and some fungi (Yoshida, 1969).

Maize shoots (200), *E. gracilis* cells (20g wet wt.), baker's yeast cells (100g wet wt.) and *P. blakesleeanus* mycelia (35g wet wt.) were incubated for 18h (maize) or 6h (*E. gracilis*, baker's yeast and *P. blakesleeanus*) under our standard conditions (Whistance *et al.* 1967; Spillor *et al.* 1968; Threlfall & Whistance, 1970) with either 4μCi of [U-^{14}C]cinnamic acid (10mCi/mmol) or 4μCi of *p*-[U-^{14}C]coumaric acid (12.9mCi/mmol) [prepared by incubating L-[U-^{14}C]phenylalanine and L-[U-^{14}C]tyrosine respectively with phenylalanine ammonia-lyase from *Rhodotorula glutinis* 1FO 559 (Ogata, Uchiyama, Yamada & Tochikura, 1967)]. At the end of the incubations the ubiquinones were isolated from the organisms and assayed for radioactivity (Whistance *et al.* 1967; Spillor *et al.* 1968; Threlfall & Whistance, 1970).

In all cases radioactivity was incorporated into ubiquinone, the major radioactive compound observed in the lipid fractions. The incorporation (percentage of dose) from [U-^{14}C]cinnamic acid varied from 0.008 in maize to 0.0005 in baker's yeast; that from *p*-[U-^{14}C]coumaric acid varied from 0.11 in baker's yeast to 0.03 in maize. The specific radioactivities (c.p.m./μmol) of the samples labelled from [U-^{14}C]cinnamic acid were 1800, 1228, 203 and 12 for maize, *E. gracilis*, *P. blakesleeanus* and baker's yeast respectively; those of the samples labelled from *p*-[U-^{14}C]coumaric acid were 4450, 5310, 2030 and 800 respectively. Ozonolytic degradations and comparison of the specific radioactivities of the ubiquinone samples with those of the 4-demethylsterols established that greater than 90% of the radioactivity in the ubiquinone molecules was in the *p*-benzoquinone ring.

The results show that in all organisms examined the *p*-hydroxybenzoic acid utilized for ubiquinone biosynthesis can be formed from *p*-coumaric acid and, with the possible exception of yeast, cinnamic acid. However, they do not rule out the possibility that some of this *p*-hydroxybenzoic acid is also formed directly from chorismic acid.

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Oxidation of Furan-2-Carboxylate to 2-Oxoglutarate by *Pseudomonas putida* F2: Studies of Enzymology and Electron Transport

By J. P. KITCHER and P. W. TRUDGILL. (*Department of Biochemistry and Agricultural Biochemistry, University College of Wales, Aberystwyth, U.K.*)

Pseudomonas putida F2 grown with furan-2-carboxylate oxidizes this compound to 2-oxoglutarate through the following sequence of intermediates: furan-2-carboxylate → 2-furoyl-CoA → 5-hydroxy-2-furoyl-CoA → 5-oxo-Δ^2-dihydro-2-furoyl-CoA → 2-oxoglutaryl-CoA → 2-oxoglutarate. A key reaction of this sequence, the hydroxylation of 2-furoyl-CoA, draws its oxygen from water with the resultant obligatory requirement for an electron acceptor, which may be satisfied by Methylene Blue or the membrane fraction from furan-2-carboxylate-grown cells (Jones & Trudgill, 1967; Trudgill, 1969).

Descriptions of other hydroxylations that draw their oxygen from water are confined to nitrogen-containing hydrophilic ring structures (Dagley &