These indicate that in crabs adapted to 850 mosmolal condition, the glutamate, proline and glycine oxidation rates are similar in terms of μmol consumed per unit time, although the lower number of C atoms in glycine means that it enables only 40% as much ADP to be phosphorylated to ATP as the other two amino acids do. When the salinity is decreased, however, the contribution of glycine C to CO_2 produced increases nearly fivefold, now being equal to glutamate and proline, even though the oxidation of both these amino acids has also increased.

Thus glycine appears to occupy a special position in terms of the provision of additional oxidizable C during adaptation to a decreased environmental salinity by *Carcinus maenas*. Preliminary data on species of *Maia* and of *Portunas* suggest that a similar situation may apply, although their tolerance to changes in external salinity is much less. The euryhaline teleost *Agonus cataphractus*, however, does not appear to increase glycine oxidation during adaptation to a lowered environmental salinity.

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Chaplin, A. E., Huggins, A. K. & Munday, K. A. (1966) *Biochem. J.* **99**, 42P–43P
Chaplin, A. E., Huggins, A. K. & Munday, K. A. (1970) *Int. J. Biochem.* **1**, 385–400
Colley, L., Rowe, W. C., Huggins, A. K., Elliott, A. B. & Dicker, S. E. (1972) *Comp. Biochem. Physiol.* **41B**, 307–322
Duchâteau, E., Florkin, M. & Jenniaux, C. (1959) *Arch. Int. Physiol. Biochim.* **67**, 489–500
Florkin, M. & Schoffeniels, E. (1969) *Molecular Approaches to Ecology*, pp. 89–111, Academic Press, New York and London
Gilles, R. (1972) *Life Sci.* **11**, 565–572
Huggins, A. K. & Munday, K. A. (1968) *Adv. Comp. Physiol. Biochem.* **3**, 271–378
King, E. N. (1965) *Comp. Biochem. Physiol.* **15**, 93–102
Needham, A. E. (1957) *Physiologia Comp. Oecol.* **4**, 209–239
Potts, W. T. W. & Parry, G. (1963) *Osmotic and Ionic Regulation in Animals*, pp. 89–224, Pergamon Press, New York and London
Shaw, J. (1955) *J. Exp. Biol.* **32**, 664–680
Shaw, J. (1958*a*) *J. Exp. Biol.* **35**, 902–919
Shaw, J. (1958*b*) *J. Exp. Biol.* **35**, 920–929
Thabrew, M. I., Poat, P. C. & Munday, K. A. (1973) *Comp. Biochem. Physiol.* **44B**, 869–879

Problem of the Reactive Species from Enzymic and Chemical Oxidations of *o*-Diphenols: Anomalies in the Trapping of *o*-Quinonoids with Benzenesulphinic Acid

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Unstable *o*-quinonoid species, formed by enzymic and chemical oxidation of *o*-diphenols, can be characterized through the formation of their stable phenylsulphonyl derivatives by reaction with benzenesulphinic acid. It is apparent, however, that some anomalies do exist in such trapping reactions. Hinsberg & Himmelschein (1896) oxidized pyrocatechol with aqueous potassium dichromate in the presence of benzenesulphinic acid. The product obtained, $C_{12}H_{10}O_4S$ (m.p. 164°C) was stated, without supporting evidence, to be the 4′-phenylsulphonyl derivative of pyrocatechol. Jackson & Koch (1901) oxidized pyrocatechol with iodine in chloroform solution in the presence of benzenesulphinic acid, and the product obtained had m.p. 153°C. Hinsberg (1903) melted together pyrocatechol and an excess of benzenesulphinic acid to obtain a phenylsulphonyl

derivative of pyrocatechol, m.p. 152–153°C. Pugh & Raper (1927) conducted the enzymic oxidation of pyrocatechol with a 'tyrosinase' preparation from mealworms, in the presence of benzenesulphinic acid and obtained a product (m.p. 164°C) identical with that obtained by Hinsberg & Himmelschein (1896).

We have studied the reactions of crystalline *o*-benzoquinone with benzenesulphinic acid in both aqueous and tetrahydrofuran solution. The product obtained from the aqueous reaction (m.p. 148–150°C) is the 4′-phenylsulphonyl catechol; that obtained from tetrahydrofuran solution (m.p. 154–156°C) is the 3′-phenylsulphonyl catechol. The trapping of other *o*-quinonoid species with benzenesulphinic acid was reviewed by Horspool (1969).

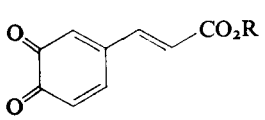
We have recently become aware of anomalies in the reactions of benzenesulphinic acid with products derived by the oxidation of caffeic acid and its derivatives. Caffeoquinone, I, and caffeoquinone methyl ester, II, were synthesized and isolated (R. Davies, unpublished work). Compounds I and II react with benzenesulphinic acid in tetrahydrofuran solution to form stable colourless products having m.p. 228–230°C and 219–220°C respectively. These products are the 5′-phenylsulphonyl derivatives of caffeic acid, III, and of methyl caffeate, IV, respectively.

In contrast, Pierpoint (1966) carried out the enzymic oxidation of chlorogenic acid with *o*-diphenol oxidase prepared from tobacco leaves, in the presence of benzenesulphinic acid. The product obtained (m.p. 170–172°C) was shown to be the 6′-phenylsulphonyl derivative of chlorogenic acid, V (Janes, 1969).

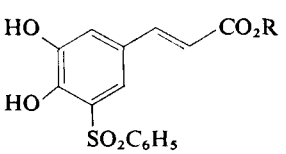
The possibility existed that the different course of reaction taken by chlorogenoquinone prepared *in situ* could be due to influence exerted by the quinic acid moiety. Therefore the enzymic oxidation of caffeic acid was carried out in the presence of benzenesulphinic acid, by using the same *o*-diphenol oxidase. The product is the 6′-phenylsulphonyl derivative of caffeic acid, VI (m.p. 215–217°C), and is identical with the product obtained by the saponification of compound V. However, reaction of crystalline caffeoquinone with benzenesulphinic acid in the aqueous buffer used for the enzymic reaction also gives the 6′-phenylsulphonyl derivative of caffeic acid, VI.

To add to an already confusing situation, Stom *et al.* (1972*a*,*b*) have also described the enzymic oxidation of caffeic acid and of chlorogenic acid in the presence of benzenesulphinic acid by a preparation of *o*-diphenol oxidase isolated from potato tuber. The product obtained from caffeic acid had m.p. 132°C, and that from chlorogenic acid had m.p. 129–130°C, both these melting points differing from those of the products we have obtained from the corresponding reactions. The authors did not assign structures but they did publish i.r. spectra. Comparisons with the published spectra are difficult, but they indicate that our preparations of compounds VI and V differ from the Russians' products from caffeic acid, and chlorogenic acid respectively.

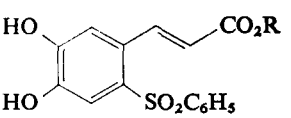
The structures of compounds III, IV, V and VI are apparent from their n.m.r. (nuclear-magnetic-resonance) spectra (Table 1). Only details of the absorptions of



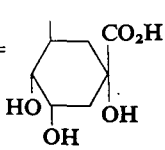
I R = H
II R = CH_3



III R = H
IV R = CH_3



V R =



VI R = H

Table 1. *N.m.r. spectra of compounds III, IV, V and VI*

Spectra were recorded at 90 MHz in d_6-acetone solution. Chemical shifts were measured in τ. s, Singlet; d, doublet; m, multiplet.

III	IV	V	VI
		1.51 d (1H) $J = 15.8$ Hz	1.47 d (1H) $J = 15.7$ Hz
1.86–2.47 m (7H)	1.88–2.50 m (7H)	2.02–2.43 m (6H)	2.03–2.49 m (6H)
2.56 d (1H) $J = 2$ Hz	2.59 d (1H) $J = 2$ Hz	2.72 s (1H)	2.72 s (1H)
3.60 d (1H) $J = 15.7$ Hz	3.62 d (1H) $J = 16$ Hz	3.87 d (1H) $J = 15.6$ Hz	3.86 d (1H) $J = 15.7$ Hz

aromatic protons and those on the side chain double bond are given. In the spectra of compounds III and IV, the doublet nature ($J = 2$Hz) of the resonance, at 2.56 and 2.59τ respectively, of one proton on the phenolic ring indicates a *meta* coupling with another aromatic proton. This shows that the phenylsulphonyl group, is substituted into the 5′ position of the phenolic ring. In the spectra of compounds V and VI, the singlet nature of the resonance, at 2.72τ in both cases, of one proton on the phenolic ring indicates that this proton lies *para* to another aromatic proton. This shows that the phenylsulphonyl group is substituted into the 6′ position of the phenolic ring in these two compounds.

These results clearly show the effect of solvent on the course of reaction, and may suggest that the enzyme also exerts such an effect. They do not eliminate the possibility that the reactive species is not the 'usual' *o*-quinone, but a semiquinone radical or 'colourless' *o*-quinone (Willstätter & Müller, 1908). The semiquinone could conceivably arise directly from the enzymic or chemical oxidation, or from the *o*-quinone.

Hinsberg, O. (1903) *Chem. Ber.* **36**, 107–115
Hinsberg, O. & Himmelschien, A. (1896) *Chem. Ber.* **29**, 2023–2029
Horspool, W. M. (1969) *Q. Rev. Chem. Soc.* **23**, 204–235
Jackson, C. L. & Koch, W. (1901) *Am. Chem. J.* **26**, 10–46
Janes, N. F. (1969) *Biochem. J.* **112**, 617
Pierpoint, W. S. (1966) *Biochem. J.* **98**, 567–580
Pugh, C. E. M. & Raper, H. S. (1927) *Biochem. J.* **21**, 1370–1383
Stom, D. I., Kalabina, A. V., Kolmakova, E. F. & Timofeeva, S S. (1972*a*) *Dokl. Akad. Nauk SSSR* **205**, 989–992
Stom, D. I., Kolmakova, E. F. & Timofeeva, S. S. (1972*b*) *Prikl. Biokhim. Mikrobiol.* **8**, 112–115
Willstätter, R. & Müller, F. (1908) *Chem. Ber.* **41**, 2580–2586

The Functioning of a Nicotinamide–Adenine Dinucleotide-Dependent Dehydrogenase and the Structure Adjacent to the Reacting Carbon Atom of the Substrate*

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Cortisone reductase (EC 1.1.1.53) from *Streptomyces hydrogenans* catalyses the transfer of the 4-pro-*S* hydrogen from NADH to C-20 of various 20-oxo steroids by a homofacial (not homochiral) reaction, forming the corresponding 20β-hydroxy compounds. Kinetic studies with derivatives of pregn-4-ene-3,20-dione led to the suggestion that the steroid-binding site of cortisone reductase is to some extent flexible, that the A ring of these bound substrates lies towards an extremity of the steroid-binding region, and that interactions between the enzyme and the β side of ring B, the region of C-11 in ring C, and the region of C-16 in ring D of the substrate are important in determining the efficiency with which the substrates are utilized. Favourable interactions were evidently hydrophobic in some regions (for example, the β side of ring B), included interactions with polar groups in other cases (for example, at C-11 in ring C), and in some cases were not capable of such simple classification (for example, in the region of C-16 and C-17 of ring D).

In pregn-4-ene-3,20-dione, the reacting carbon atom (C-20) is the carbonyl carbon of the 17β-acetyl side chain. The enzyme can, however, accommodate a bigger side chain than this, for example a 17β-*O*-acetylglycollyl side chain. The polarity of the side chain affected the kinetic parameters, but in some respects was not highly critical, the carbonyl group of a 17β-glycollyl side chain and a 17β-acetyl side chain, for example, both under-

* Not presented at the Meeting.