The Importance of (Non-enzymic) Chemical Reaction Processes to the Fate of Foreign Compounds in Mammals

By D. E. Hathway
IMPERIAL CHEMICAL INDUSTRIES LIMITED, CENTRAL TOXICOLOGY LABORATORY, ALDERLEY PARK, CHESHIRE SK10 4TJ

1 Introduction

The study of foreign compound metabolism in mammals developed contemporaneously with that of intermediary biochemistry and by the time of World War I some examples of the main types of biotransformation that occur had been discovered. They included the oxidations of benzene and toluene, respectively, to phenol and benzoic acid,[1] the reduction of nitro-groups, for example of 3-nitrobenzaldehyde to afford 3-acetylaminobenzoic acid,[2] and the hydrolysis of esters, as well as the conjugation of alcohols, carboxylic acids, phenols, and thiols with glucuronic acid,[3] the conjugation of benzoic acids with glycine[4,5] and of phenylacetic acid (in man) with glutamine,[6] the conjugation of phenol with sulphuric acid[7] and of bromo-, chloro-, and iodo-benzenes with mercapturic acid.[8–10] In addition, the *N*-methylation of pyridine (acetate)[11] and the low-yield biotransformation[12] of CN^- into SCN^- were known. The idea began to emerge that foreign compound metabolism in mammals might confer a protective function, and in Neumeister's[13] 'Lehrbuch der physiologische Chemie' the word 'Entgiftung (detoxication)' was coined. However, on the basis of the metabolism of the diverse foreign substances, with which mammals might be treated or to which they might be exposed, conversion into compounds of greater activity, *i.e.* 'bioactivation'[14] would appear to be similarly feasible, and Young,[15]

[1] O. Schultzen and B. Naunyn, *Arch. Anat. Physiol.*, 1867, 349.
[2] R. Cohn, *Z. physiol. Chem.*, 1893, **17**, 274.
[3] O. Schmiedeberg and H. Meyer, *Z. physiol. Chem.*, 1879, **3**, 422.
[4] W. Keller, *Annalen*, 1842, **43**, 108.
[5] O. Schultzen and C. Gräbe, *Arch. Anat. Physiol.*, 1867, 166.
[6] H. Thierfelder and C. P. Sherwin, *Ber.*, 1914, **47**, 2630.
[7] E. Baumann, *Pflügers Arch. ges. Physiol.*, 1876, **13**, 285.
[8] E. Baumann and C. Preusse, *Ber.*, 1879, **12**, 806.
[9] E. Baumann and P. Schmitz, *Z. physiol. Chem.*, 1895, **20**, 586.
[10] M. Jaffe, *Ber.*, 1879, **12**, 1092.
[11] W. His, *Arch. exp. Path. Pharmakol*, 1887, **22**, 253.
[12] S. Lang, *Arch. exp. Path. Pharmakol*, 1894, **34**, 247.
[13] R. Neumeister, 'Lehrbuch der physiologischen Chemie mit Berücksichtigung der pathologischen Verhältnisse für Studierende und Ärzte (1st edn.)' Jena, 1895, Vol. 11 p. 346.
[14] E. J. Ariens and A. M. Simonis, in 'Molecular Pharmacology', ed. E. J. Ariens, Academic Press, New York, 1964, Vol. 1, p. 77.
[15] L. Young, *Physiol. Rev.*, 1939, **19**, 323.

Stekol,[16] and Williams[17,18] drew attention to the possible problems accruing to the use of the term 'detoxication'. By the early 1960's, the discovery of the drug-metabolizing enzymes, and especially of the cytochrome P-450 system associated with the smooth endoplasmic reticulum and of the cytosol enzymes, imposed order on the study of foreign compound metabolism.[19,20] It then became clear that the biotransformation of foreign compounds into less lipid-soluble products, which would be more readily excretable from the body, might well be the physiological function of the drug-metabolizing enzymes, and that such a concept paralleled earlier hypotheses concerning the surface tension[21] and increased acidity[22] of the polar conjugates excreted.

Throughout the development of this field of knowledge, the operation of biochemical reactions had been tacitly assumed, and the establishment of enzyme-catalysed mechanisms both for the reactions of foreign compounds and for intermediary metabolites strongly supported this supposition. Nevertheless, review of the last two decades' literature[23] brought to the author's attention some metabolites, which were unlikely to result from biotransformations alone. This paper is not a review in the usually accepted sense but rather a discussion paper in which the author explores the possibility that non-enzymic reactions may play a significant role in foreign compound metabolism in mammals.

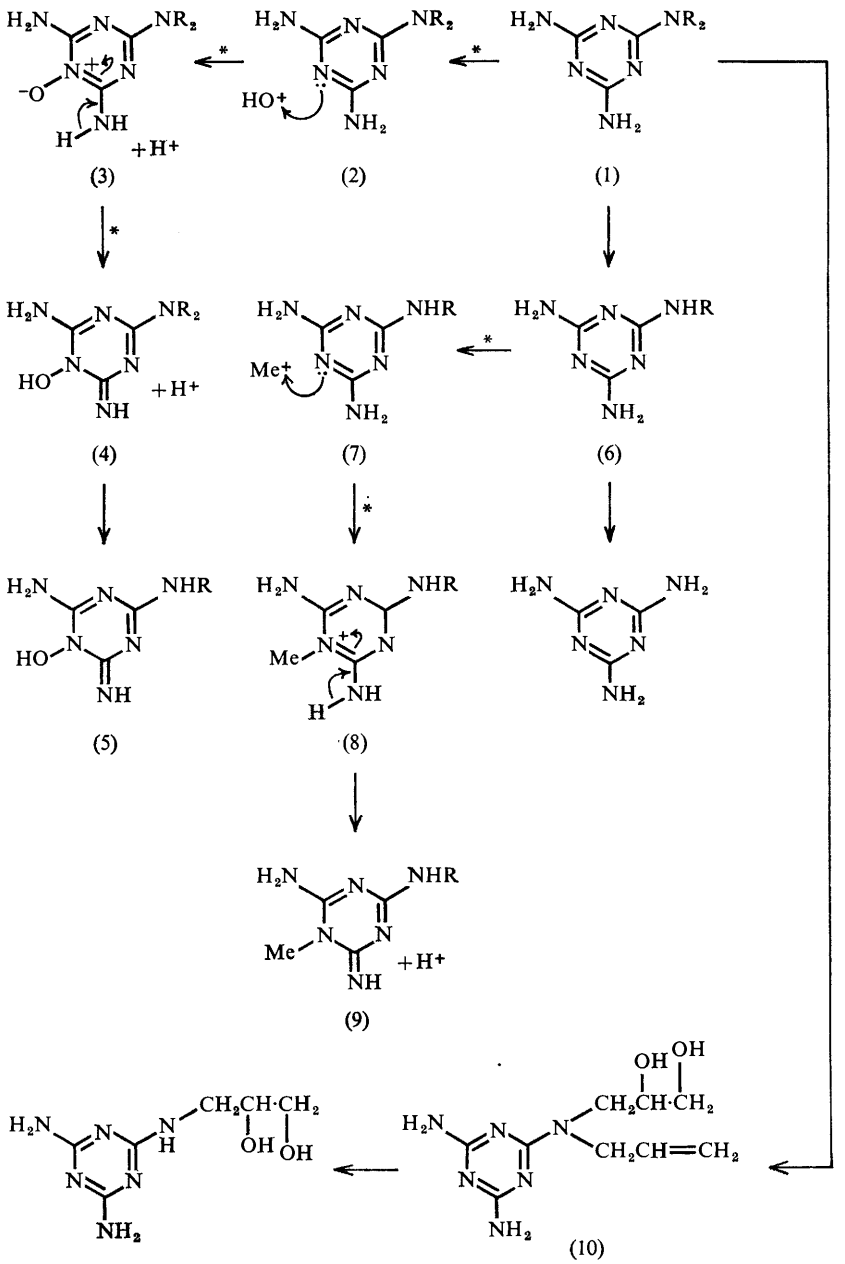
2 The Method

Methodology used for detecting and testing chemical reactions suspected of contributing to the biological fate of a foreign compound is illustrated by reference to *NN*-diallylmelamine (1) (Scheme 1, where $R{=}CH_2{\cdot}CH{=}CH_2$).

The possibility of chemical reaction processes contributory to foreign compound metabolism may be detected by the unusual structures of identified metabolites and by the unlikelihood of their arising from enzyme-catalysed reactions: such chemical reaction steps may be accompanied by bioactivation. It ought to be stated clearly at the outset of this discussion that many of the reaction steps in this and the subsequent schemes (*q.v.*) should be regarded as speculative or reasonable chemical possibilities. The suggested reaction steps, for which there is no supporting experimental evidence, are marked with an asterisk. Thus, in a preliminary investigation of the properties of the vasodilator (1), Zins[24] found in dogs and rats, but not in man, 'a unique *N*-oxidation product (4)', which was twenty-one times more hypotensive than (1).

The suggested reaction steps must account for the orientation and origin of the substances concerned, and ought to be compatible with physiological conditions

[16] J. A. Stekol, *Ann. Rev. Biochem.*, 1941, **10**, 265.
[17] R. T. Williams, 'Detoxication mechanisms', 1st edn., Chapman & Hall, London, 1947.
[18] R. T. Williams, 'Detoxication mechanisms', 2nd edn., Chapman & Hall, London, 1959.
[19] B. B. Brodie and E. G. Erdös, 'Proc. 1st Internat. Pharmacol. Meeting (1961)', Pergamon Press, Oxford, 1962, Vol. 6, Metabolic Factors Controlling Duration of Drug Action, p. 1.
[20] J. R. Gillette, *Fortschr. Arzneim.-Forsch.*, 1963, **6**, 11.
[21] L. Berczeller, *Biochem. Z.*, 1917, **84**, 75.
[22] A. J. Quick, *J. Biol. Chem.*, 1932, **97**, 403.
[23] 'Foreign Compound Metabolism in Mammals', ed. D. E. Hathway (Specialist Periodical Reports), The Chemical Society, London, 1970—1979, Vols. 1—5.
[24] G. R. Zins, *J. Pharmacol.*, 1965, **150**, 109.



* Indicates **reaction steps** for which there is no experimental evidence.

Scheme 1

and with the overall product analysis for the foreign compound under scrutiny.

Thus, (i) from the chemical standpoint, (1) (Scheme 1) and its *N*-de-allylation product (6) appear to undergo electrophilic substitution, which would account for the orientation and origin of Zins'[24] unique oxidation product (4), of the (4) *N*-de-allylation product (5), and of the ring-methylation product (9). It is envisaged that (4) results by electrophilic attack of HO^+ at the ring –N= of (1) followed by proton loss, and in consequence the mechanism (1)—(4) for electrophilic substitution of the *sym*-triazine ring has now been inserted (Scheme 1) into the metabolic pathway of Zins *et al.*[25] for (1). Similarly, (9) results by electrophilic attack of Me^+ from a biological methylating agent at the ring –N= of (6) followed by proton loss, and again the mechanism (6)—(9) has been inserted into the metabolic pathway.[25] In both of these cases, substitution occurs at the ring nitrogen atom that is shown and not at the other theoretically possible ones, as this is the only ring nitrogen atom which is located between two primary-amine ring-substituents.

(ii) The question of energy changes for the chemical reactions is important as such steps ought to be compatible with physiological conditions. However, an extensive literature search revealed that none of the energy changes for the reactions with which this discussion is concerned had been measured. In addition, relevant data cannot be obtained by molecular orbital calculation because of molecular complexity and because of the absence of appropriate reference compounds. Any attempt to relate these energy changes to resonance energies for classical structures (*inter al.* Dewar and Trinajstić[26]) is bound to incur approximation and, for example, in the case of the *sym*-triazines would have involved specific tautometers, which are unlikely to exist; the resulting values would thus be seen to have no firm scientific basis. The point of view is reached in the present communication that simple chemical steps, like the ones postulated (1)—(4) and (6)—(9), which would be expected to take place under gentle reaction conditions or which are analogous to reactions that do, are likely to occur *in vivo*: this was essentially Robinson's idea[27,28] about biosynthetic processes (see also Collie[29,30]).

(iii) The proportion of products resulting from (1) by key chemical reactions depends upon the reaction rates for these processes relative to those for competing biochemical reactions. The preferred side-chain hydroxylation route *via* (10) and the *N*-de-allylation route *via* (6) account for 90% of an acute dose, and the relatively low formation of ring-hydroxylated metabolites (4), (5) appears to agree with this supposition. The ring *N*-methylation product (9) accounts for a small proportion only of the (1) de-allylation products.

(iv) By corollary, a chemical reaction *in vivo* ought to be unaffected by the physiological processes of induction and inhibition of the hepatic drug-metaboliz-

[25] G. R. Zins, D. E. Emmert, and R. A. Walk, *J. Pharmacol.*, 1968, **159**, 194.
[25] M. J. S. Dewar and N. Trinajstić, *Theoret. Chim. Acta*, 1970, **17**, 235.
[27] R. Robinson, *J. Chem. Soc.*, 1917, **111**, 762.
[28] R. Robinson, *J. Chem. Soc.*, 1917, **111**, 876.
[29] J. N. Collie, *J. Chem. Soc.*, 1893, **63**, 329.
[30] J. N. Collie, *J. Chem. Soc.*, 1907, **91**, 1806.

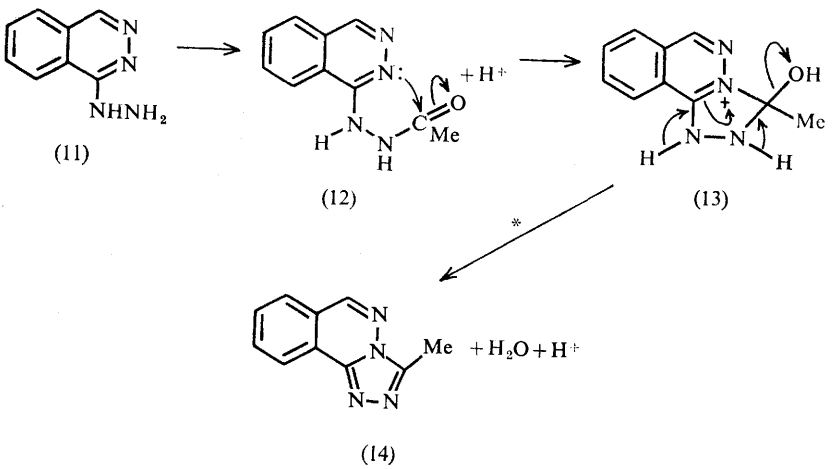
ing enzymes. SKF525A markedly inhibited side-chain hydroxylation of (1) *via* (10) and oxidative *N*-de-allylation of (4) to give (5), and it moderately inhibited oxidative *N*-de-allylation of (1) *via* (6) including ring *N*-methylation by the same route.[25] But, predictably, a low degree of *N*-oxidation of the *sym*-triazine ring *via* electrophilic substitution (1)—(4) was unaltered by SKF525A (see Scheme 1).

In addition, some mention ought to be made of the gross variation in media which foreign compounds meet (in the body) from pH 0 to 8, from oxidizing to reducing environments, from high to low concentrations of Ca^{2+}, and the variety of surfaces to which they will be exposed. However, the extremes of this range of variation do not apply to the body fluids and tissues with which the present paper is concerned, but the possibility of mechanisms in colloidal solutions ought not to be disregarded.

3 Selected Drugs and Pesticides

The biological fate of some other drugs and pesticides is examined for contributory chemical reaction processes by the previously described analysis as far as experimental evidence is available.

The principal urinary metabolite of the vasodepressor, hydralazine, 1-hydrazinophthalazine (11) (Scheme 2), in animals[31,32; *cf.*33] and in man[34] is



Scheme 2

methyl-*sym*-triazolo[3,4-*a*]phthalazine (14). It is now suggested that the product of (11) bioacetylation, 1-(2-acetylhydrazino)phthalazine (12), undergoes simul-

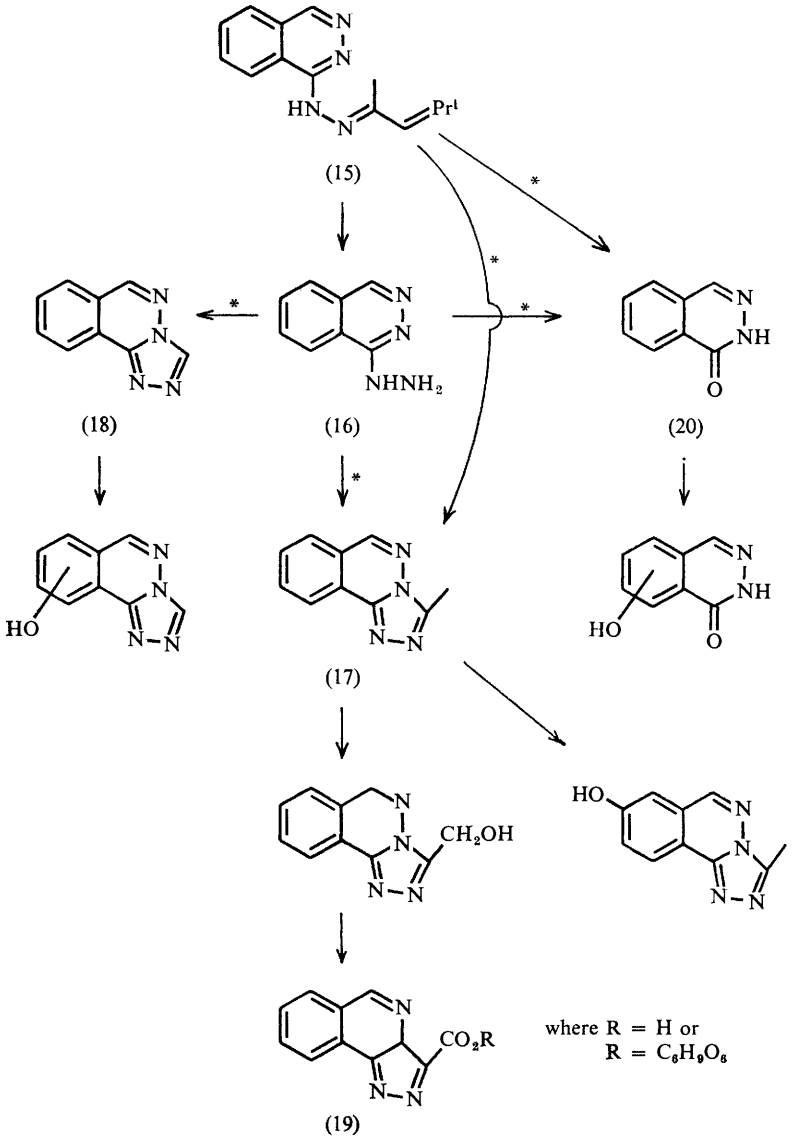
[31] S. Edwards and F.-H. Marquardt, *Z. physiol. Chem.*, 1969, **350**, 85.
[32] H. Zimmer, J. McManus, T. Novinson, E. V. Hess, and A. H. Litwin, *Arzneim.-Forsch.*, 1970, **20**, 1586.
[33] W. M. McIsaac and M. Kanda, *J. Pharmacol.*, 1964, **143**, 7.
[34] H. Zimmer, J. Kokosa, and D. A. Gartiez, *Arzneim.-Forsch.*, 1973, **23**, 1028.

taneously proton-induced enolization of the acetyl C=O and ring-closure followed by proton loss plus loss of the elements of water (12)—(14). It is not known[34] whether (11) or (14) contributes to the serious side effects, *viz* the production of an acute, sub-acute, or chronic syndrome that is indistinguishable from *lupus erythematosus*. However, the suggested reaction sequence does account for the orientation and origin of (14). The rate-limiting step for its synthesis *in vivo* seems to be the bioacetylation of (11), as non-enzymic acetylation with acetyl CoA affords a very small amount of (14).[35] As additional supporting chemical evidence for the structure, the prior synthesis of 3-methylimidazo[5,1-*a*]isoquinoline by acetylation of 1-aminomethylisoquinoline[36] was cited.[32]

Budralazine, 1-[2-(1,3-dimethyl-2-butenylidene)hydrazino]phthalazine (15) (Scheme 3), a derivative of hydralazine (16) (*v. supra*), affords the metabolites 3-methyl-*sym*-triazolo[3,4-*a*]phthalazine (17) and *sym*-triazolo[3,4-*a*]phthalazine (18).[37,38] Tracer studies[38] established that in the metabolism of (15), (18) was synthesized by formylation of (16) with formate generated from a C_1-unit of tetrahydrofolic acid, and not by decarboxylation of *sym*-triazolo[3,4-*a*]phthalazine-3-carboxylic acid (19; where R = H). On the other hand, metabolism of (15)-d_{10} in rats proved[38] that (17) was formed not only *via* (16), where the methyl group of (17) originated from the acetyl group introduced by bioacetylation, but also to a very limited extent by direct oxidative ring-closure of (15), where the methyl group came from the butylidene group of (15) *per se*. It is reasonable to suppose that the mechanistic arguments that were invoked for the metabolism of hydralazine (16) into (17) (*v. supra*) apply as well to that of (15) into (17) and (18) *via* (16). Rate-limiting steps for the synthesis of all seven *sym*-triazolo[3,4-*a*]-phthalazine metabolites appear to be the hydrolysis of the keto-hydrazone (15) itself and the subsequent bioacylations of (16). Another point might be mentioned. In their diagrammatic representation of (15) metabolism (Scheme 3), Moroi *et al.*[38] claim that (15) and (16) are both converted into (20), but careful examination of their work[37,38] does not reveal supporting experimental evidence for this supposition. As far as is known, (20) has never been identified as a metabolite of (16). In any case, the small yield of (20), only 1—2% of a dose of (15) in two strains of rat,[37] suggests that (15) and (16) act as poor substrates for an oxidative deaminase.

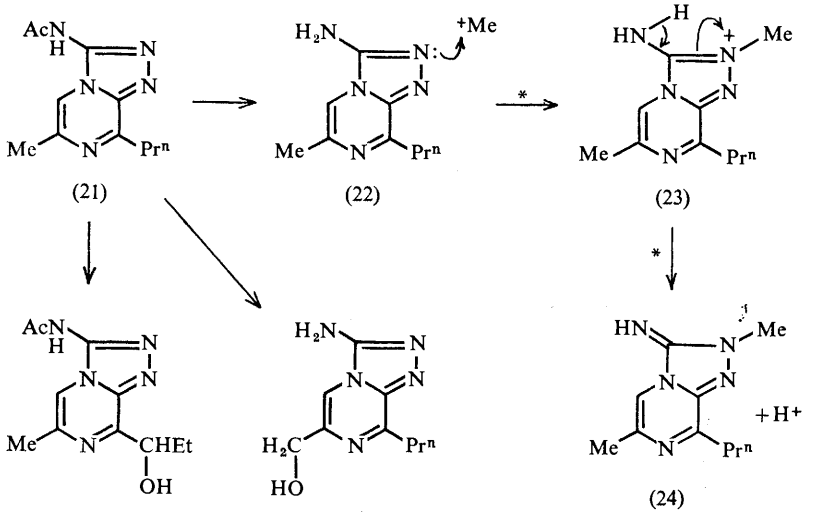
The drug 3-acetamido-6-methyl-8-n-propyl-*sym*-triazolo[4,3-*a*]pyrazine (21) (Scheme 4), which is used for the treatment of asthma, afforded in mammals a metabolite with 'a somewhat unusual structure' (24).[39] It is now suggested that the product of deacetylase action, 3-amino-6-methyl-8-n-propyl-*sym*-triazolo-

[35] D. E. Drayer, J. M. Strong, B. Jones, A. Sandler, and M. M. Reidenberg, *Drug Metabolism and Disposition*, 1974, **2**, 499.
[36] H. Zimmer, D. G. Glasgow, M. McClanahan, and T. Novinson, *Tetrahedron Letters*, 1968, 2805.
[37] R. Moroi, K. Ono, T. Saito, M. Sano, and T. Akimoto, *Chem. and Pharm. Bull. (Japan)*, 1976, **24**, 2850.
[38] R. Moroi, K. Ono, T. Saito, T. Akimoto, and M. Sano, *Chem. and Pharm. Bull. (Japan)*, 1977, **25**, 830.
[39] D. E. Case, R. S. McDonald, and H. Illston, *Xenobiotica*, 1972, **2**, 45.



Scheme 3

[4,3-*a*]pyrazine (22), undergoes electrophilic substitution. In fact, (24) is perceived to result from electrophilic attack of Me^+ from a biological methylating agent at the ring –N= of (22), followed by proton loss. The mechanism (22)—(24) has

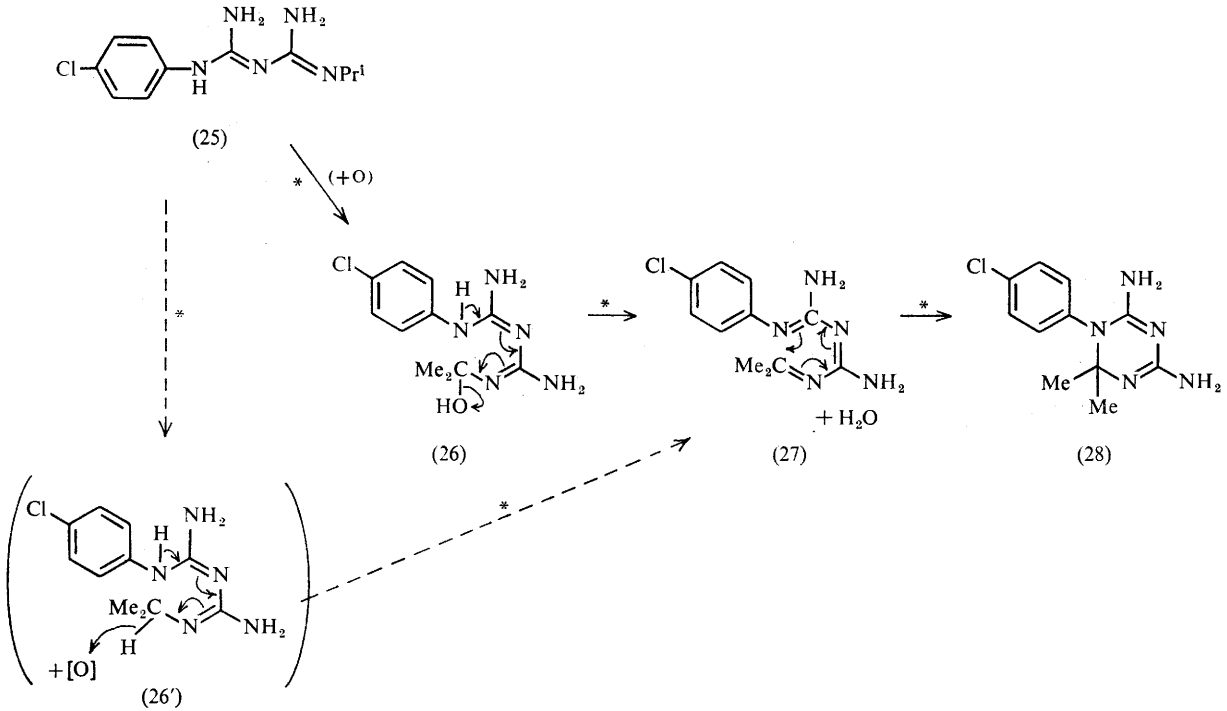


Scheme 4

been inserted into the metabolic pathway of Case *et al.*[39] Up to 60% of a dose of (21) was metabolized into (22) in dogs (*ca.* 70% in rats), and the greater conversion into (24) in dogs [21% of a dose of (21)] than in rats (7%) is attributed to the favourable competition of methylation with the biochemical conjugation of (22) in this species. If dogs were treated with (22), which eliminated hydroxylation of the 6-methyl and 8-n-propyl substituents of the parent drug (21), the proportion of (24) increased to 40%.

In the human metabolism of proguanil (25) (Scheme 5), Carrington *et al.*[40,41] and Crowther and Levi[42] detected the substituted dihydro-*sym*-triazine metabolite (28), which is a ten times more powerful antimalarial than original (25). There is a strong supposition that cytochrome P-450 mediated ($\omega - 1$) oxidation of (25) would give a reaction intermediate (26) which, by elimination of the elements of water *via* the cyclic mechanism represented, followed by reverse cyclization of the bonds with ring-closure (27), would afford (28). Alternatively, although less likely in the author's opinion, the hydroxylation to (26) might be obviated by involving the attack of oxene at the ($\omega - 1$) site of (25), followed by a rather similar elimination of the elements of water from (26′) to that postulated in the previous mechanism, whence (28) would result through reverse cyclization of the bonds with ring-closure. (Oxene is written as [O] and is isoelectronic with the now classical carbenes and nitrenes).

[40] H. C. Carrington, A. F. Crowther, D. G. Davey, A. A. Levi, and F. L. Rose, *Nature*, 1951, **168**, 1080.
[41] H. C. Carrington, A. F. Crowther, and G. J. Stacey, *J. Chem. Soc.*, 1954, 1017.
[42] A. F. Crowther and A. A. Levi, *Brit. J. Pharmacol.*, 1953, **8**, 93.

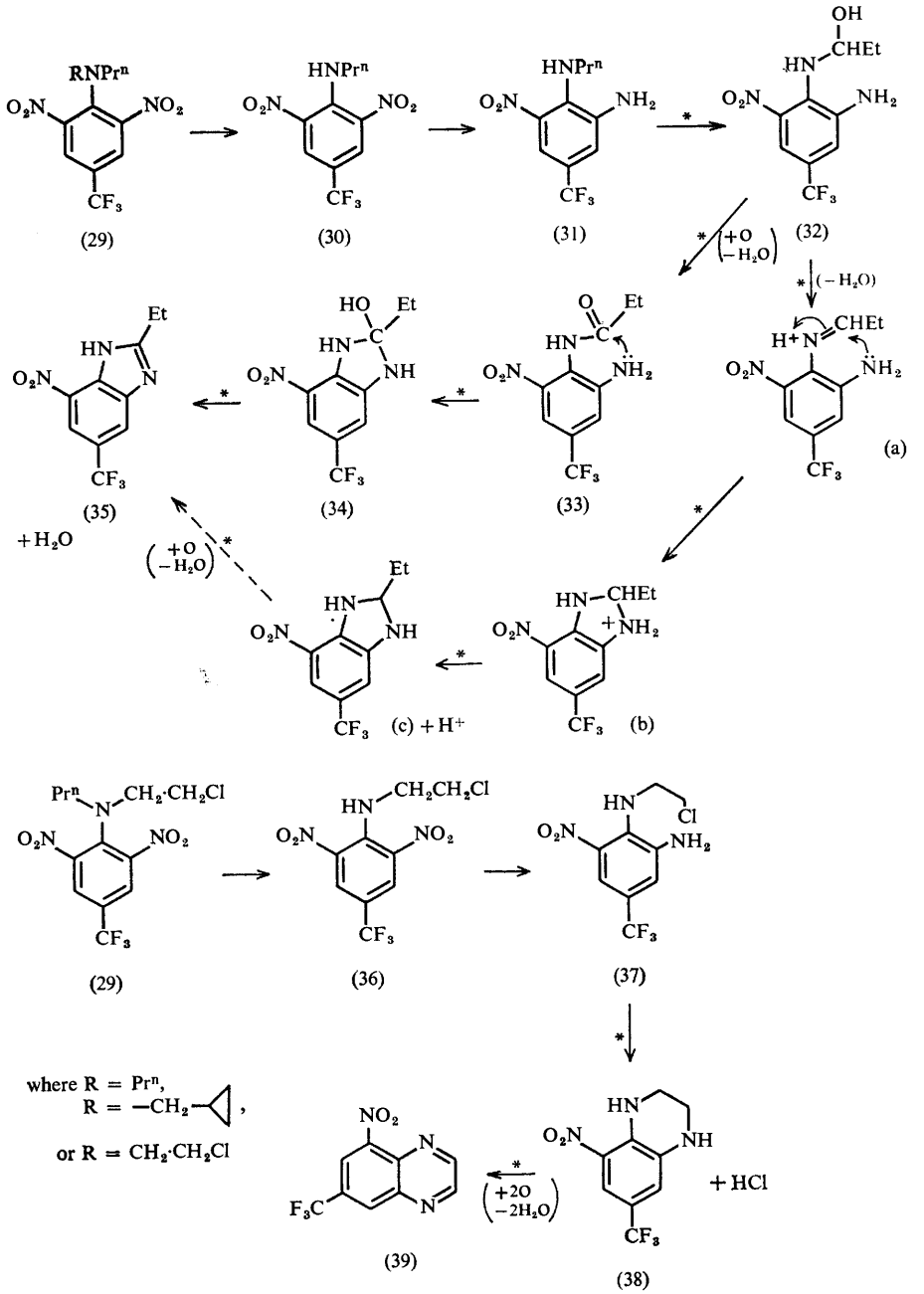

NH2 NH2
Cl
N
H
N
NPr^i
(25)
* (+O)
*
Cl
H NH2
N
N
Me2C
N
HO
NH2
(26)
*
Cl
NH2
N
C
N
Me2C
N
NH2
+ H2O
(27)
*
Cl
NH2
N
N
Me
Me
N
NH2
(28)
Cl
H NH2
N
N
Me2C
H
N
NH2
+[O]
(26′)
*

Scheme 5

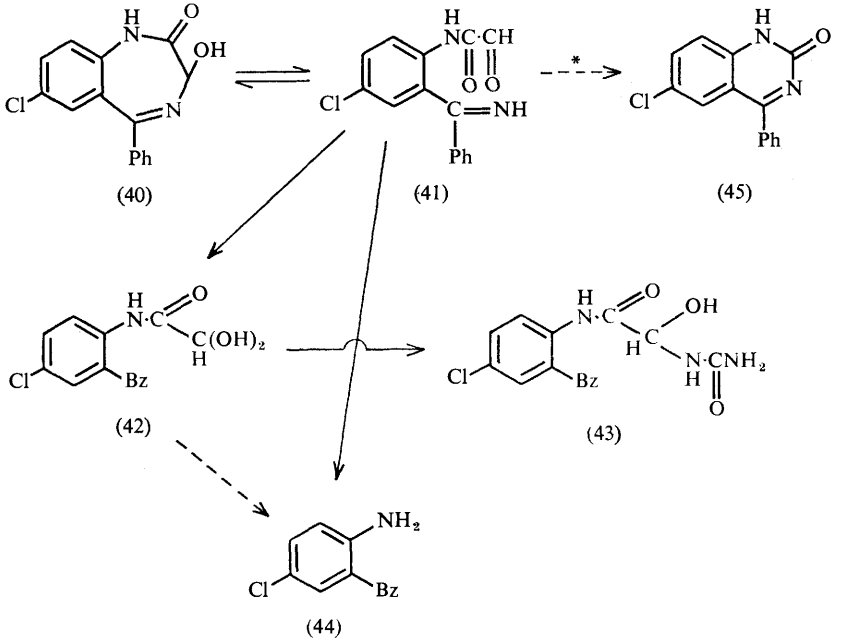
Other examples of unusual ring-closure are provided by the metabolism of the substituted 2,6-dinitroaniline herbicides, trifluralin (29; R = Pr^n), profluralin (29; R = CH_2–cyclopropyl), and fluchloralin (29; R = CH_2CH_2Cl (Scheme 6).[43] Since all three substances afford a common *N*-de-alkylation product (30) and another metabolite with a reduced nitro-group, it is reasonable to suspect that (31) might be the starting material for benzimidazole (35) biosynthesis. There is a strong supposition that cytochrome P-450 mediated oxidation of (31) would afford a reaction intermediate (32), as such substances have been isolated as well known intermediates in oxidative *N*-de-alkylations. Whence, ring-closure of the corresponding amide (33) oxidation product, rather than of (32) *per se* which would have led (32)—(a)—(b) to the dihydrobenzimidazole (c), would afford the benzimidazole (35) itself *via* dehydration of the *gem*-hydroxy reaction intermediate (34), and this appears to be the preferred mechanism. However, enzymic dehydrogenation of the dihydrobenzimidazole product (c) by the other route is not ruled out.[43a] It is further suggested that another unusual metabolite of (29; R = CH_2CH_2Cl), the substituted quinoxaline (39), might be derived by nitro-reductase from the known metabolite (36), followed successively by loss of the elements of hydrogen chloride with ring-closure and by aromatization.

The tranquillizer oxazepam (40) exemplifies ring-fission (Scheme 7). Three out of six minor metabolites of (40) in man are open-ring compounds,[44] and open-ring intermediates are considered to participate also in the synthesis of a fourth metabolite which exhibits ring-contraction. The suggestion that (40) appears to show imino–enamine ring–chain tautomerism[45–47] would account for the origin of all four metabolites, (42) – (45). Thus, hydrolysis of the imino-group belonging to the open-ring form (41) would furnish the key metabolite, 2′-benzoyl-4′-chloro-2,2-dihydroxyacetanilide (42), which demonstrates aldehydic properties in its conjugation with glucuronic acid and in the formation of a urea adduct (43), which is itself another metabolite. Glyoxals of the type RCO·CHO, to which (42) belongs, readily form hydrates. The third metabolite, 2-amino-5-chlorobenzophenone (44), is a hydrolytic product of (41)—(43). It is probable that 6-chloro-4-phenyl-2(1*H*)quinazolinone (45) is formed from (40) *via* the open-ring form (41), but the mechanism is entirely unknown. However, oxidation is suspected to occur for one carbon atom to be lost as CO_2, but feasible involvement of a carbamic acid reaction intermediate with a great propensity for decarboxylation is unacceptable as such a reaction intermediate, if formed, is unlikely to last long enough to be implicated in the cyclization envisaged. The corresponding pyruvic acid, for which these arguments do not apply, would cyclize to give a substituted

[43] J. O. Nelson, P. C. Kearney, J. R. Plinner, and R. E. Menzer, *Pesticide Biochem. Physiol.*, 1977, **7**, 73.
[43a] R. W. Chadwick, L. T. Chuang, and K. Williams, *Pesticide Biochem. Physiol.*, 1975, **5**, 575.
[44] S. F. Sisenwine, C. O. Tio, S. R. Shrader, and H. W. Ruelius, *Arzneim.-Forsch.*, 1972, **22**, 682.
[45] S. C. Bell and S. J. Childress, *J. Org. Chem.*, 1962, **27**, 1691.
[46] L. H. Sternbach, E. Reeder, A. Stempel, and A. I. Rachlin, *J. Org. Chem.*, 1964, **29**, 332.
[47] S. C. Bell, R. J. McCaully, C. Gochman, S. J. Childress, and M. I. Gluckman, *J. Medicin. Chem.*, 1968, **11**, 457.


RNPr^n
HNPr^n
HNPr^n
OH
CHEt
HN
O_2N
NO_2
NH_2
CF_3
(29)
(30)
(31)
(32)
(+O, $-H_2O$)
($-H_2O$)
(a)
HO
Et
O
HN
C
NH
NH_2
(33)
(34)
(35)
$+H_2O$
(+O, $-H_2O$)
CHEt
H^+
N
HN—CHEt
$+NH_2$
(b)
(c) $+H^+$
Pr^n
$CH_2{\cdot}CH_2Cl$
CH_2CH_2Cl
Cl
(29)
(36)
(37)
where R = Pr^n,
R = $-CH_2-$cyclopropyl,
or R = $CH_2{\cdot}CH_2Cl$
(+2O, $-2H_2O$)
(39)
(38)
$+HCl$

Scheme 6



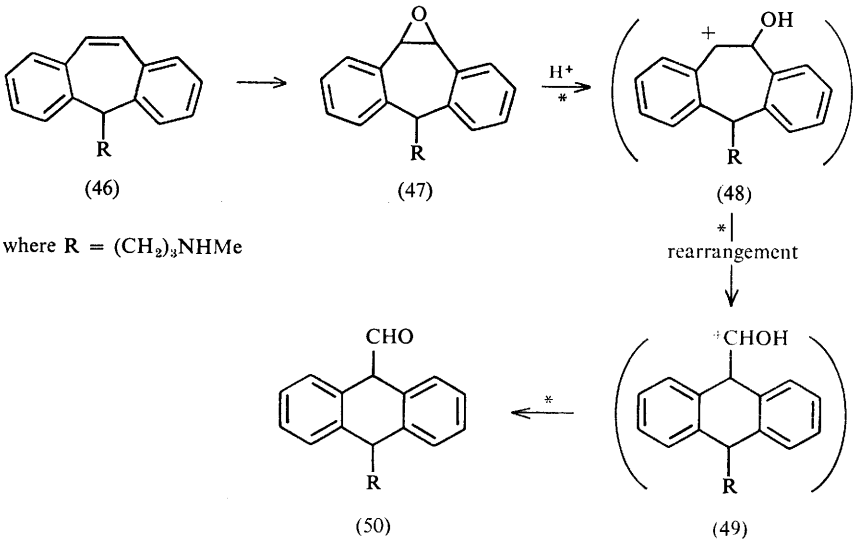
Scheme 7

quinazoline carboxylic acid. Moreover, condensation of (44) with urea is apparently unfavourable,[44] since biosynthesis of (45) does not occur in animals dosed with (44). Metabolites (42)—(45) seem to be formed by non-enzymic chemical steps, as the proportions in which they are formed are unaltered by induction of the drug-metabolizing enzymes *in vivo*, and the product analysis is consistent with slow reaction rates for the formation of (42)—(45).

Ring-contraction (*v. supra*) seems to be a rather unusual metabolic process, and one of the very few cases that has been reported[48] is the alleged biotransformation of protriptyline (46) (Scheme 8) into 3-(10-formyl-9,10-dihydroanthracen-9-yl) propyl-*N*-methylamine (50) by pinacol-type rearrangement (47)—(50) of the epoxide (47). However, as these authors[48] used acid in their isolation procedure, (50) might be an artifact. More than 40% of a dose of (46) has now been found[49] in fact to be excreted as epoxide products that were resistant both to epoxide hydratase and to the glutathione, glutathione *S*-epoxide transferase system. Whether any of (50) was formed from (46) *in vivo* has yet to be established.

It has been suggested[50] that D-homoannulation (Scheme 9) of 17α-ethynylo-

[48] S. F. Sisenwine, C. O. Tio, S. R. Shrader, and H. W. Ruelius, *J. Pharmacol.*, 1970, **175**, 51.
[49] H. B. Hucker, A. J. Balletto, J. Demetriades, B. H. Arison, and A. G. Zacchei, *Drug Metabolism and Disposition*, 1975, **3**, 80.
[50] M. T. Abdel-Aziz and K. I. H. Williams, *Steroids*, 1969, **13**, 809.



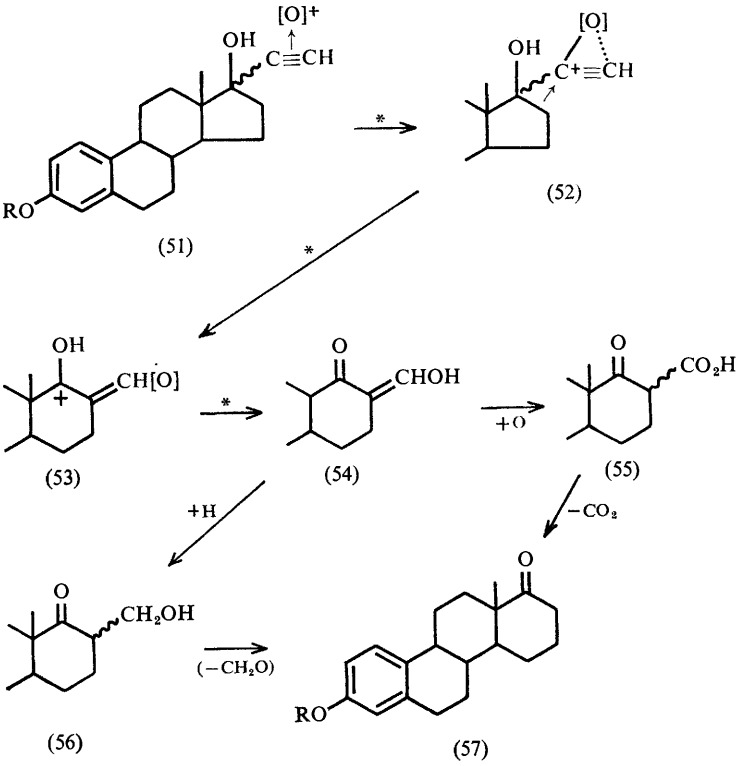
Scheme 8

estradiol and its 3-methyl ether occurs *in vivo* by a mechanism involving biochemical oxidation and a concerted pinacol-type rearrangement suggested by analogy with previous chemical studies,[51] followed by further chemical reaction processes. Attack of an oxidizing enzyme on the electron-rich triple bond of (51) is considered to afford a molecular species (52) with a partial positive charge on C-20, whence migration of the 16—17 bond in a pinacol-type rearrangement and collapse of the intermediate (53) would give 17-hydroxymethylene-D-homo-oestr-17α-one (54). Either oxidation of (54) to (55) or reduction to (56) would then result in ready loss of the original C-21 carbon atom to give D-homo-oestr-17α-one (57). Mechanistic arguments for D-homoannulation of (51) apply as well to that of norgestrel (13β-ethyl-18,19-dinor-17α-pregn-4-en-20-yn-3-one) enantimorphs in women.[52,53]

4 Survey of Some Industrial Chemicals

As chemical reaction processes have been shown to be important to the biological fate of the foregoing drugs and pesticides, they may play a more extensive role in the metabolism and biological interactions of foreign compounds than had previously been suspected, and in this connexion the fate of some industrial chemicals is accordingly now explored.

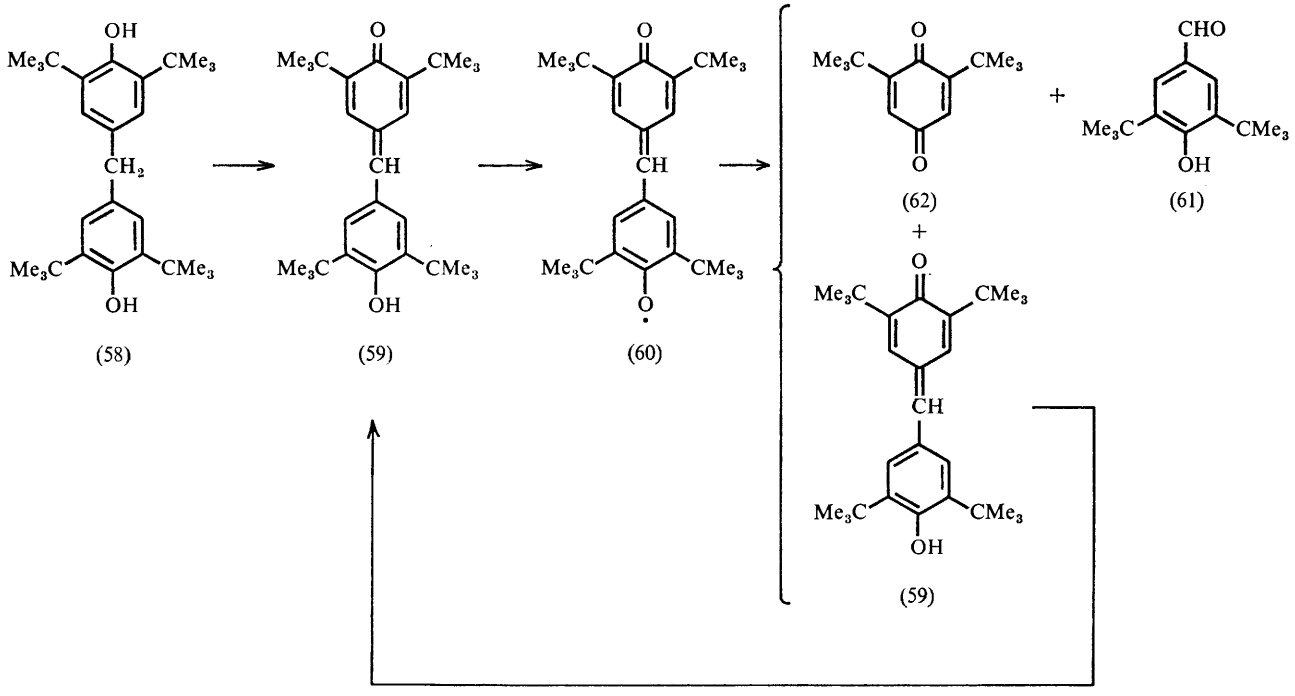
[51] K. I. H. Williams, M. Smulowitz, and D. K. Fukushima, *J. Org. Chem.*, 1965, **30**, 1447.
[52] S. T. Sisenwine, H. B. Kimmel, A. L. Liu, and H. W. Ruelius, *Acta endocrinol.*, 1973, **73**, 91.
[53] S. T. Sisenwine, H. B. Kimmel, A. L. Liu, and H. W. Ruelius, *Drug Metabolism and Disposition*, 1975, **3**, 180.



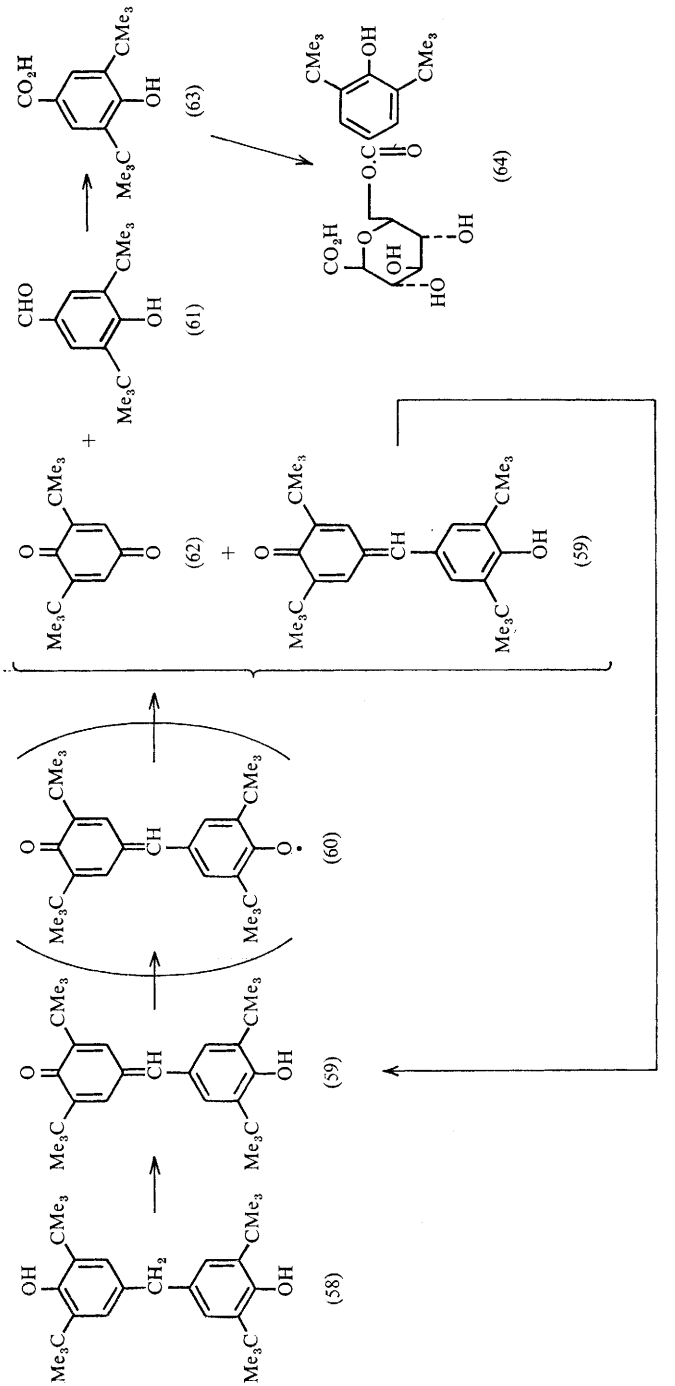
Scheme 9

The fact that some rather unusual metabolites of hindered phenolic antioxidants *in vivo* are closely related to the expected products of antioxidant action *in vitro* seems to suggest that they may result from chemical reaction processes. Thus, antioxidant action of Ionox 220, di-(3,5-di-t-butyl-4-hydroxyphenyl) methane (58) (Scheme 10), might be represented by the reaction sequence (58)—(62),[54,55] leading through the quinone methide 3′,5′-di-t-butyl-4′-hydroxyphenyl-(2,6-di-t-butyl-*p*-benzoquinone)methide (59) to Coppinger's[56] stable phenoxy-radical (60), two molar proportions of which dismutate to give (59), 3,5-di-t-butyl-4-hydroxybenzaldehyde (61), and 2,6-di-t-butyl-*p*-benzoquinone (62).[57] The metabolic products, which include the quinone methide (59), (62), 3,5-di-t-butyl-4-hydroxybenzoic acid (63) (Scheme 11), and its ester β-D-glucuronide (64),[54,55] are strikingly similar to the foregoing products of antioxidant action. The 'metabolic pathway' for (58) seems to comprise the chemical reaction

[54] D. E. Hathway, *Adv. Food Res.*, 1967, **15**, 1.
[55] A. S. Wright, R. S. Crowne, and D. E. Hathway, *Biochem. J.*, 1966, **99**, 146.
[56] G. M. Coppinger, *J. Amer. Chem. Soc.*, 1957, **79**, 501.
[54] M. S. Kharasch and B. S. Joshi, *J. Org. Chem.*, 1957, **22**, 1439.


OH
Me3C
CMe3
CH2
Me3C
CMe3
OH
(58)
O
Me3C
CMe3
CH
Me3C
CMe3
OH
(59)
O
Me3C
CMe3
CH
Me3C
CMe3
O
(60)
O
Me3C
CMe3
O
(62)
+
CHO
Me3C
CMe3
OH
(61)
+
O
Me3C
CMe3
CH
Me3C
CMe3
OH
(59)

Scheme 10

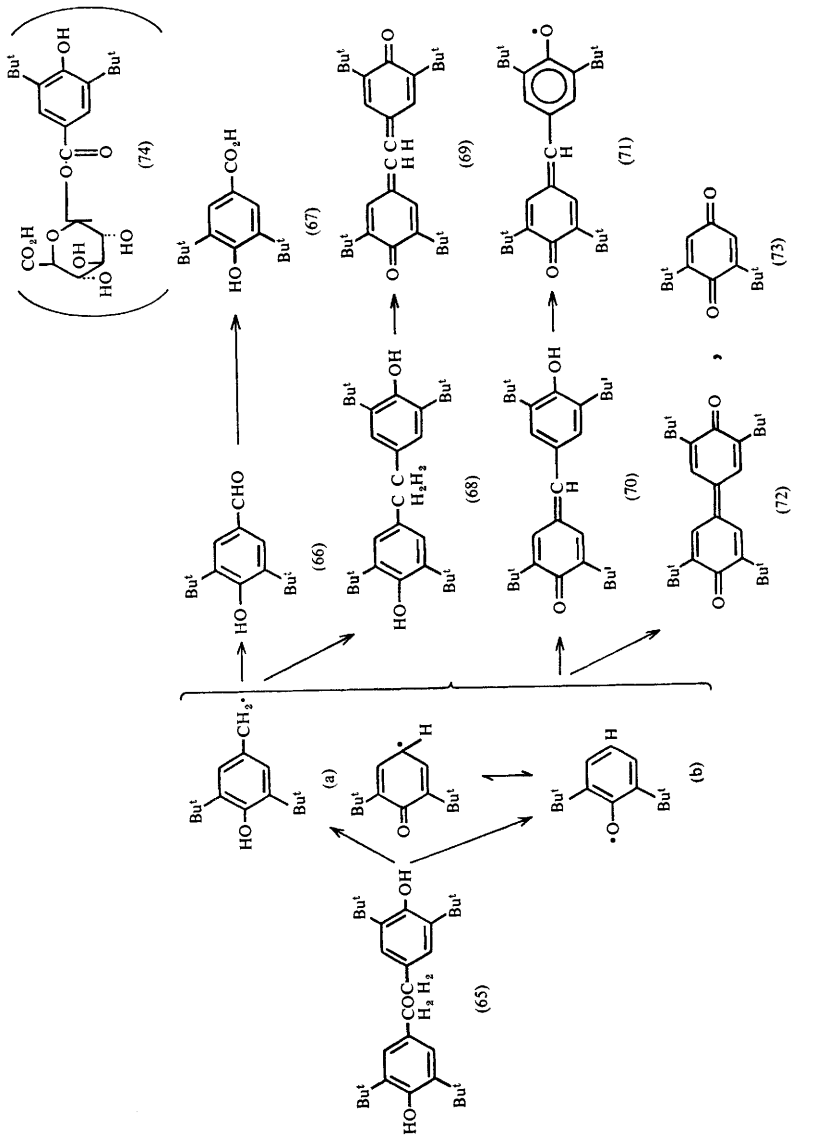


Scheme 11

sequence (58)—(62), which contributes to antioxidant action, and also the biochemical oxidation of (61) into (63) and glucuronic acid conjugation of (63). Thus, low-energy chemical reaction processes account for the orientation and origin of Ionox 220 metabolites. The metabolism of Ionox 201, di-(3,5-di-t-butyl-4-hydroxybenzyl)ether (65) (Scheme 12)[54,58] *in vivo* also appears to be closely related to its antioxidant action *in vitro*. The antioxidant action of (65), which involves a radical mechanism, was simulated by reaction with a single-electron oxidizing agent, lead dioxide.[58] Besides the Ionox 201 metabolites 3,5-di-t-butyl-4-hydroxybenzaldehyde (66), 3,5-di-t-butyl-4-hydroxybenzoic acid (67), 3,3′,5,5′-tetra-t-butyl-4,4′-stilbenequinone (69), and the quinone methide (70), the following compounds were produced, *viz* di-(3,5-di-t-butyl-4-hydroxybenzyl) (68), Coppinger's[56] stable phenoxy-radical (71), 3,3′,5,5′-tetra-t-butyl-4,4′-diphenoquinone (72), and 2,6-di-t-butyl-*p*-benzoquinone (73). All of the compounds (66)—(73), but not the ester β-D-glucuronide (74), can be derived from two radicals,[59] (a) and (b) (Scheme 12), generated in the oxidation reaction. Moreover, because of the method of isotopic labelling of (65), (72) and (73) would not have been detected in the investigation *in vivo*. It follows that the presence of (66), (69), and (70) in the faeces and (66) in the adipose tissues of animals dosed with Ionox 201 probably arose in the two biological situations by chemical reactions involving radical mechanisms.

At this stage in the discussion, it should be clear that the scientific basis of each suspected contribution of chemical reactions to foreign compound metabolism must be examined carefully. Thus, the alleged tautomerism of 2-naphthylamine in aqueous solution to afford a low proportion of the imino-form,[60] which is supposed to account[60] for the action of flavoprotein oxidase on 2-naphthylamine but not on 1-naphthylamine, may be unnecessary. In fact, there is a considerable difference in basicity between 1- and 2-naphthylamines (*inter al.* Guha and Zuhradník[61]), which correlates with the conjugative effect of the unsubstituted aromatic nucleus (Robinson's[62] inductive effect), facilitating delocalization of the lone pair of electrons on the nitrogen atom of 1-naphthylamine, and the preference of the oxidase for 2-naphthylamine is likely to correspond to an enzyme specificity for a substrate with the dissociation properties of that amine. Moreover, the aromaticity of the unsubstituted nucleus would act against enamine–imino tautomerism in the other nucleus. In any case, other enzymes can bring about *N*-hydroxylation of 2- and probably also of 1-naphthylamines, and the considerations required by flavoprotein oxidase do not apply to cytochrome P-450.[63,64] The question as to which of these enzyme systems effects *N*-hydroxylation of the naphthylamines *in vivo* has never been resolved. Even basic ideas

[58] A. S. Wright, R. S. Crowne, and D. E. Hathway, *Biochem. J.*, 1966, **102**, 351.
[59] S. L. Cosgrave and W. A. Waters, *J. Chem. Soc.*, 1951, 388.
[60] L. L. Poulson, B. S. S. Masters, and D. M. Ziegler, *Xenobiotica*, 1976, **6**, 481.
[61] S. Guha and R. Zahradník, *Coll. Czech. Chem. Comm.*, 1967, **32**, 2448.
[62] R. Robinson, 'Outline of an electrochemical (electronic) theory of the course of organic reactions', The Institute of Chemistry of Great Britain and Ireland, London, 1932.
[63] H. Uehleke, *Xenobiotica*, 1971, **1**, 327.
[64] H. Uehleke, *Drug Metabolism and Disposition*, 1973, **1**, 299.



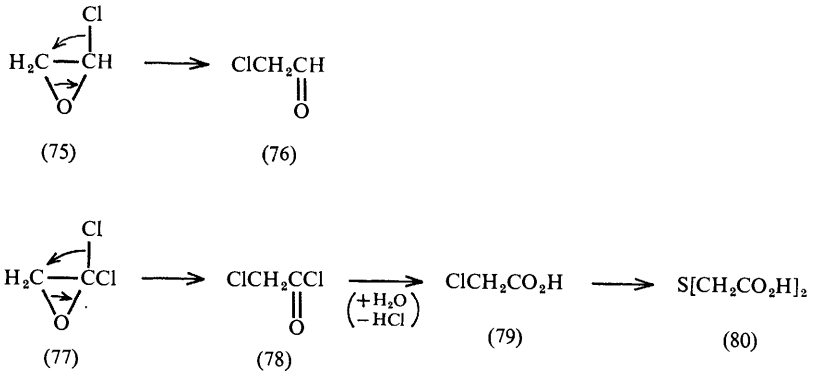
Scheme 12

about the carcinogenicity of the naphthylamines are confused and, when the first epidemiological data became available and early tests were made in animals, commercial 1-naphthylamine contained 4—10% of 2-naphthylamine. But pure 1-naphthylhydroxylamine was mutagenic in one test system[65] and not in another one,[66] whereas if 1-naphthylamine were non-carcinogenic, then 1-naphthylhydroxylamine and the parent amine would be expected to have given a consistently negative response in tests of this sort. In another approach, carcinogenicity to the dog bladder seems to correlate with the combined excretion of nitrosoarene plus arylhydroxylamine, in that 2-naphthylamine gives more of these oxidation products than the same amount of 1-naphthylamine; this observation implies that 2-naphthylamine is a more potent carcinogen than its positional isomer, and this conclusion agrees with the facts.

Outside of the immediate field of foreign compound metabolism, the possibility that chemical reaction processes may occur *in vivo* has been implied occasionally. Thus, the supposition that the imino-form of 2-naphthylamine behaves as a substrate for flavoprotein oxidase rests on the assumption of enamine–imino tautomerism under the prevailing conditions (*v. supra*). Other illustrations stem from investigation (*inter al.* Ross[67]) of the chemical reactivity in relation to the carcinogenic potential of direct alkylating agents, for example mustard gas [di-(2-chloroethyl)sulphide], nitrogen mustard HN2 [di-(2-chloroethyl)methylamine], dimethyl sulphate, ethyl and methyl methanesulphonates, β-propiolactone, glycidol, and the phosphates like cyclophosphamide, which implies that these substances react *in vivo* in much the same way as they do in aqueous solution *in vitro*. But, even in these cases,[67] it now seems unlikely that the implications of the occurrence *in vivo* of chemical reaction processes, as distinct from biochemical ones, were fully recognized.

That the expected intermediates of oxidative attack on the halogeno-alkenes are in fact halogeno-epoxides[68–73] has been substantiated recently in the case of vinyl chloride and vinylidene chloride, respectively, by the work of Green and Hathway,[74] and by that of Walker and Hathway,[75] and Jones and Hathway[76] *in vivo*. Thus, reaction of vinyl chloride-derived chloroethylene oxide (75) (Scheme 13) or its rearrangement product, chloroacetaldehyde (76), with calf-thymus DNA at pH 4.5 gave a modified DNA, which afforded amongst the products of enzymic hydrolysis 9β-D-2′-deoxyribofuranosylimidazo[2,1-*i*]purine ('etheno-deoxyadenosine') and 1β-D-2′-deoxyribofuranosyl-1,2-dihydro-2-oxo-imidazo[1,-

[65] B. Heineman, *Appl. Microbiol.*, 1971, **21**, 726.
[66] T. H. Corbett, C. Heidelberger, and W. F. Dove, *Mol. Pharmacol.*, 1970, **6**, 667.
[67] W. C. J. Ross, 'Biological alkylating agents', Butterworths, London, 1962.
[68] J. F. Powell, *Brit. J. Ind. Med.*, 1945, **2**, 142.
[69] J. W. Daniel, *Biochem. Pharmacol.*, 1963, **12**, 795.
[70] K. C. Leibman, *Mol. Pharmacol.*, 1965, **1**, 239.
[71] K. H. Byington and K. C. Leibman, *Mol. Pharmacol.*, 1965, **1**, 247.
[72] K. C. Leibman and W. J. Allister, *J. Pharmacol.*, 1967, **157**, 574.
[73] G. Bonse, Th. Urban, D. Reichert, and D. Henschler, *Biochem. Pharmacol.*, 1975, **24**, 1829.
[74] T. Green and D. E. Hathway, *Chem.-Biol. Interactions*, 1978, **22**, 211.
[75] G. H. Walker and D. E. Hathway, *Biochem. J.*, 1977, **167**, 505.
[76] B. K. Jones and D. E. Hathway, *Chem.-Biol. Interactions*, 1978, **20**, 27.

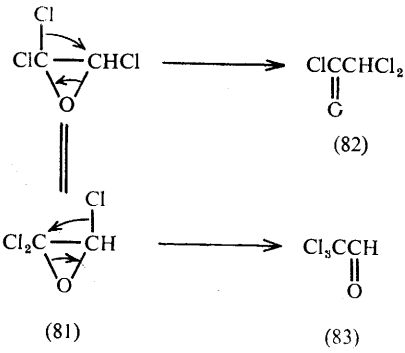


Scheme 13

2-*c*]pyrimidine ('etheno-deoxycytidine'), which were also found in the enzymic hydrolysates of the modified DNA extracted from the liver of rats that had been exposed to vinyl chloride in their drinking water.[74] In the case of vinylidene chloride (1,1-dichloroethylene), it was found[76] that, irrespective of whether [1-^{14}C]dichloroethylene or [1-^{14}C]chloroacetic acid (79) was administered to rats, 1 mol equiv. of the resulting [^{14}C]thiodiglycollic acid (80) yielded by electrolysis approximately 1 mol equiv. of $^{14}CO_2$, which is interpreted in terms of the labelling of one of the carboxylic acid groups of thiodiglycollic acid.[75] This evidence is consistent with the transformation of vinylidene chloride into chloroacetic acid (79) by a mechanism which involved migration of one chlorine atom and the loss of the other one, and this holds only where the halogeno-alkene is converted into a corresponding halogeno-epoxide (77), which rearranges into chloroacyl chloride (78). In each of the foregoing cases, rearrangement of the biochemically generated halogeno-epoxide is a chemical reaction process and the rearrangement products form sequences with a complex series of subsequent biochemical reactions.[76–79]

Of the theoretically possible mechanisms for rearrangement of 1,1,2-trichloroethylene-derived 1,1,2-trichloroethylene oxide (81) (Scheme 14), the one involving migration of hydrogen is unlikely,[80,81] and the mechanism leading to dichloroacetyl chloride (82) through rearrangement of chlorine, which involves CO heterolysis and implicates a carbonium ion destabilized by a directly bound chlorine atom, provides the preferred reaction process. But a synchronous mechanism[82] cannot be excluded altogether. Alternative rearrangement of (81) into chloral (83) proceeds through a carbonium ion with two directly bound

[77] T. Green and D. E. Hathway, *Chem.-Biol. Interactions*, 1975, **11**, 545.
[78] T. Green and D. E. Hathway, *Chem.-Biol. Interactions*, 1977, **17**, 137.
[79] B. K. Jones and D. E. Hathway, *Brit. J. Cancer*, 1978, **37**, 411.
[80] R. N. McDonald and R. N. Steppel, *J. Amer. Chem. Soc.*, 1969, **91**, 782.
[81] K. Griesbaum, R. Kibar, and B. Pfeffer, *Annalen*, 1975, 214.
[82] G. Köbich, J. Grosser, and W. Werner, *Chem. Ber.*, 1973, **106**, 2610.

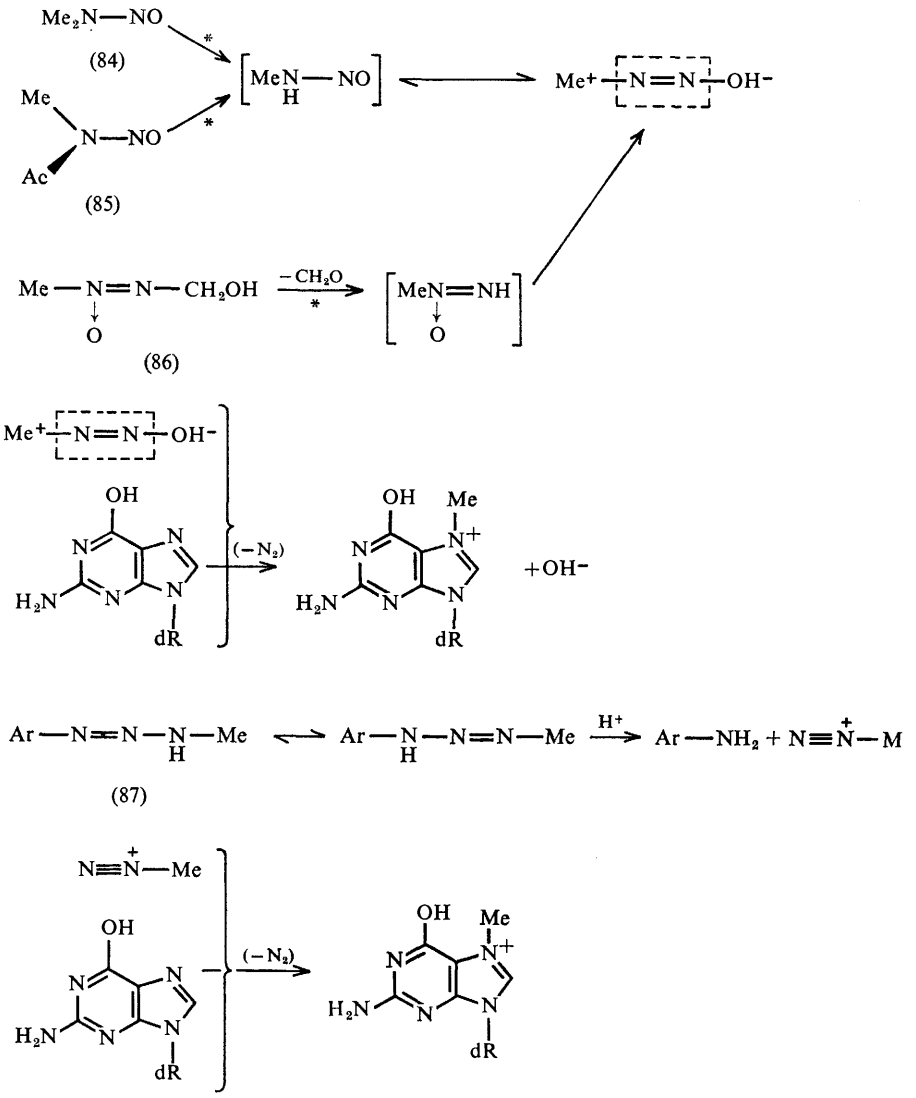


Scheme 14

chlorine atoms and is less favoured. The fact that (83) is the authenticated rearrangment product *in vivo*, although in aqueous solution *in vitro* (82)-derived dichloroacetic acid is formed exclusively, suggested to Hathway[83] that 'the usual rearrangement of (81) into (83)' may occur in hepatocyte smooth endoplasmic reticulum (SER) in contact with a catalytic centre simulating a Lewis acid. Fast cytosol biotransformation of (83) would facilitate its egress from the SER. In mice, on the other hand, rearrangement of (81) into (83) appears to be hindered. In that species, greater cytochrome P-450 activity in the liver and kidneys[84] would favour a faster rate of epoxidation, and there would be a tendency for the catalytic sites for chloroalkene oxide rearrangement to become saturated as well as for the lower activity of the cytosol enzymes responsible for (83) disposition[71,85] to become rate-limiting. Under these circumstances, (83) would accumulate in the vesicles, and in a situation where the substrate is in excess with respect to the enzyme in murine hepatocytes *in vivo*, the rest of the (81) produced would be transformed into (82) at the cytosol surface. The presence of dichloroacetic acid as well as trichloroacetic acid in the urine of treated mice strongly supports a spill-over model for 1,1,2-trichloroethylene metabolism in mice.[83]

Nucleoside residues of the nucleic acids and amino-acid residues of proteins react chemically with the electrophilic reagents formed through activation in the body of such chemical carcinogens as the dialkylnitrosamines (84) (Scheme 15), the alkyl nitrosamides (85), methylazoxymethanol (86) from cyclasin, and the alkylaryltriazenes (87). In line with the ideas of Miller and Miller,[86] deoxyguanosine reacted both *in vitro* and *in vivo* with those centres of the 2-fluorenylacetamide derivative, *viz* at the amido-nitrogen and at C-3, which were activated through esterification of the *N*-hydroxylation product.[87] Thus, gentle chemical reaction

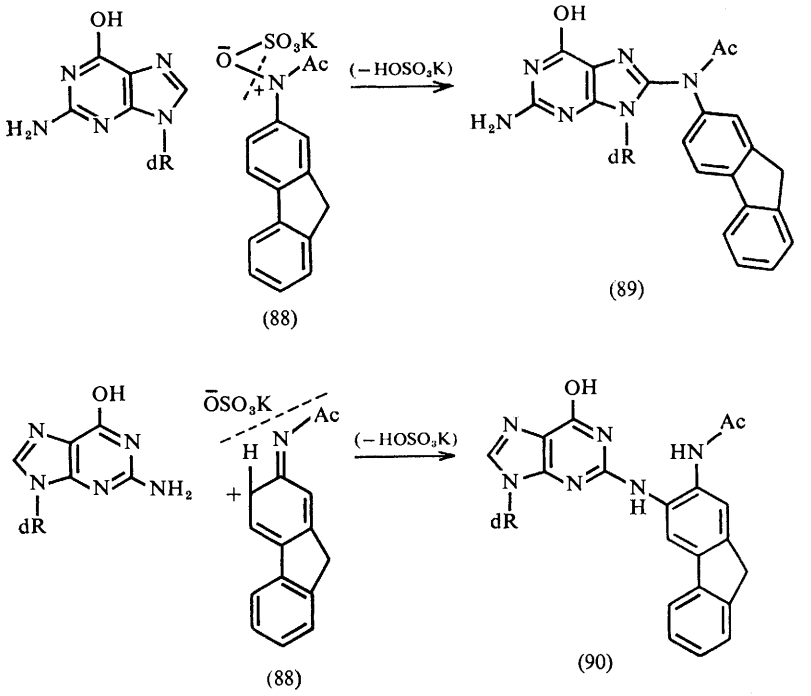
[83] D. E. Hathway, *Cancer Letters*, 1979, **8**(3), 263.
[84] C. L. Litterst, E. C. Minnaugh, R. L. Reagan, and T. E. Gram, *Drug Metabolism and Disposition*, 1975, **3**, 259.
[85] E. M. Waters, H. B. Gerstner, and J. E. Huff, *J. Toxicol. Environ. Health*, 1976–1977, **2**, 671.
[86] J. A. Miller and E. C. Miller, *J. Natl. Cancer Inst.*, 1971, **47**, v.
[87] J. G. Westra, E. Kriek, and H. Hittenhausen, *Chem.-Biol. Interactions*, 1976, **15**, 149.



Scheme 15

between deoxyguanosine and the ultimate carcinogen of 2-fluorenylacetamide, *viz* *N*-sulphonoxy-*N*-2-fluorenylacetamide (88) (Scheme 16), gave (i) *N*-(deoxyguanosin-8-yl)-2-fluorenylacetamide (89),[87] which had been identified previously as a reaction product by Kriek *et al.*,[88] and (ii) 3-(deoxyguanosin-N^2-

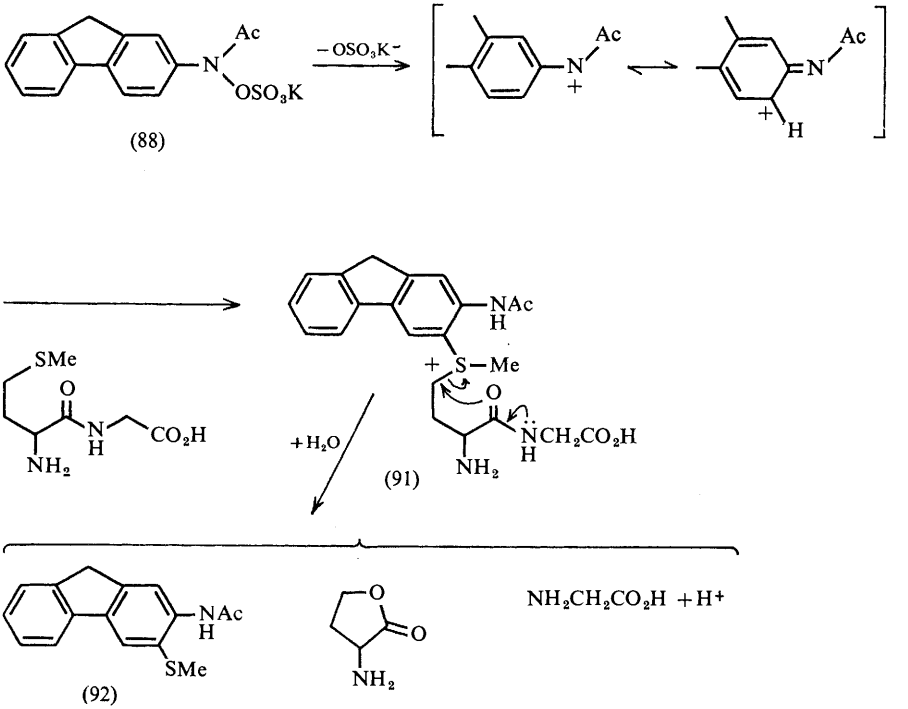
[88] E. Kriek, J. A. Miller, U. Juhl, and E. C. Miller, *Biochemistry*, 1967, **6**, 177.



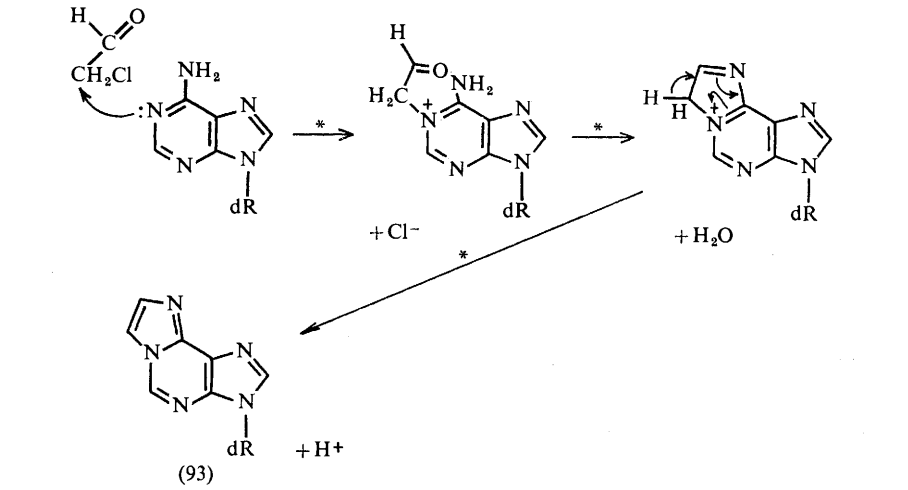
Scheme 16

yl)-2-fluorenylacetamide (90), identical with the product obtained from hydrolysates of the modified DNA extracted from the livers of rats that had been injected with *N*-hydroxy-2-fluorenylacetamide.[87] *N*-Sulphonoxy-*N*-2-fluorenylacetamide (88) also reacts chemically with methionyl peptides (Scheme 17), the adducts of which (91) decompose readily to yield 3-methylmercapto-2-fluorenylacetamide (92) and its 1-positional isomer, which were also obtained from the liver protein of rats fed *N*-hydroxy-*N*-2-fluorenylacetamide.[89] The Millers and their co-workers[86,87,89] have made extensive use of S_N1 mechanisms involving carbenium and nitrenium ions (Schemes 15—17). Formation of 9β-D-2′-deoxyribofuranosylimidazo[2,1-*i*]purine (93) (Scheme 18) and 1β-D-2′-deoxyribofuranosyl-1,2-dihydro-2-oxo-imidazo[1,2-*c*]pyrimidine by reaction of vinyl chloride-derived chloroethylene oxide or its rearrangement product, chloroacetaldehyde, with calf-thymus DNA, and from liver DNA extracted from rats that had been exposed chronically to vinyl chloride[74] implied a common reaction mechanism. There is a strong supposition that in both cases initial alkylation occurs at the ring –N= (N-1 of the deoxyadenosine residues and N-3

[89] P. D. Lotikar, J. D. Scribner, J. A. Miller, and E. C. Miller, *Life Sci.*, 1966, **5**, 1263.



Scheme 17



Scheme 18

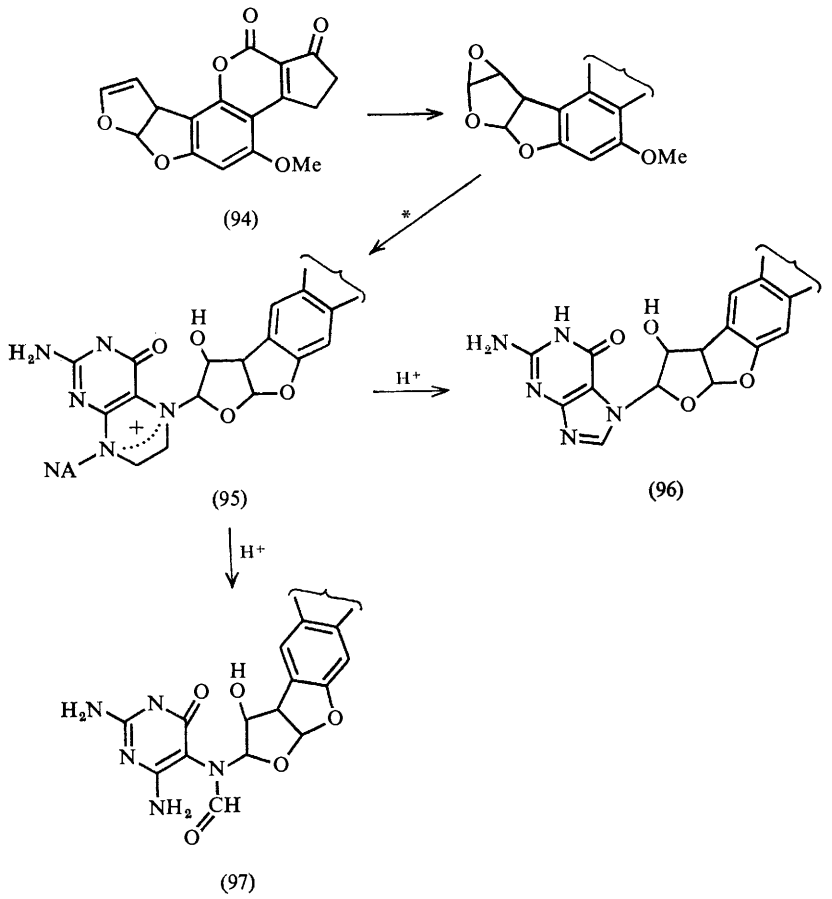
of the deoxycytidine residues), followed successively by loss of the elements of water with ring-closure between the oxygen function and the amino group belonging to C-6 of the deoxyadenosine residues (and to C-4 of the deoxycytidine residues) and by proton loss. Chloroacetaldehyde also reacted *in vitro* and *in vivo* with cysteinyl peptides to give modified peptides, which eventually afforded the urinary vinyl chloride metabolites, *viz* thiodiglycollic acid and *N*-acetyl-*S*-(2-hydroxyethyl)cysteine, by a sequence of interrelated biochemical reactions.[78] Imidazo-cyclization of the DNA deoxyadenosine and deoxycytidine residues by vinyl chloride metabolites *in vivo*[74] is reminiscent of that brought about by the chemical reaction of glycidaldehyde with the N-1 and C-2 amino-group of guanosine or deoxyguanosine.[90] Formation of the nucleoside analogues concerned (Scheme 18) is not a simple alkylation process (*cf.* Schemes 15—17).

The structure of the DNA- and ribosomal RNA-bound aflatoxin B_1 (94) (Scheme 19) adducts (95), obtained[91] from salmon sperm DNA and rat-liver ribosomal RNA with fortified rat- and hamster-liver microsomes, was inferred by hydrolysis, which yielded 2,3-dihydro-2-(guan-7-yl)-3-hydroxyaflatoxin B_1 (96) as the major product and 2,3-dihydro-2-(N^5-formyl-2,5,6-triamino-4-oxopyrimidin-N^5-yl)-3-hydroxyaflatoxin B_1 (97) as a minor one. Chemical reaction between 2-position of the aflatoxin B_1-epoxy metabolite and 7-position of the nucleic acid deoxyguanosine and guanosine residues thus took place both *in vitro* and *in vivo*.[91]

Finally, it ought to be stated clearly that no attempt has been made to treat the title subject exhaustively, but the author has attempted to draw attention to some areas of foreign compound metabolism, where the occurrence of chemical reaction processes *in vivo* might be important to the exposed mammal. Thus, chemical reaction processes have been shown to produce drug metabolites with greater pharmacological activity than the original substances *per se*, and *NN*-diallylmelamine and proguanil exemplify this possibility. In some of the other examples of drugs and pesticides that were cited, unusual metabolites may contribute to the spread of biological activity associated with the parent substances. Formation of new agents by chemical reaction processes would seem to oppose the defence of organs and tissues afforded by the cysteinyl peptides and by the drug-metabolizing enzymes of the SER. This idea is particularly relevant to carcinogens that are activated through metabolism and which were considered towards the end of the present narrative. In fact, the biological potential of a particular carcinogen for a tissue must take account of the efficacy of alkylating reactions for the determinative genome macromolecule and of the detoxication of the alkylating metabolites occurring there.

In retrospect, a mechanistic approach to suspect chemical reactions *in vivo* may be simplistic and may have incurred in turn some error in detail. However, this is a discussion paper that is exploratory in concept (*v. supra*). The mechanisms concerned have been the subject of considerable debate, but Alexander

[90] B. M. Goldschmidt, T. P. Balzej, and B. L. Van Duuren, *Tetrahedron Letters*, 1968, 1583.
[91] Jen-kun Lin, J. A. Miller, and E. C. Miller, *Cancer Res.*, 1977, **37**, 4430.



Scheme 19

Pope's famous dictum 'Survey the whole, nor seek slight fault to find' is respectfully requested in this case as well.

5 Conclusion

It appears: (i) that in mammals treated with a foreign compound, purely chemical reactions may occur in solution, if they are mechanistically favoured; (ii) that the chemical reactions of a foreign compound and its metabolites give products that are eliminated from the body along with those of accompanying biotransformations, or that interact with enzyme-catalysed reactions; (iii) that an appropriate 'metabolic pathway' unites all of the simultaneously occurring biochemical and chemical reaction processes. Plausible chemical reactions appear to account for the orientation and origin of a number of substances which have been

scrutinized in the present communication and appear to be consistent with physiological conditions and the product analyses for the foreign compounds concerned. The fate of a few drugs and pesticides *in vivo* served to demonstrate electrophilic substitution in different heterocyclic sytems, various ring-closure mechanisms, ring-fissions exemplified by imino–enamine ring–chain tautomerism, as well as ring-contraction *via* an open-ring form (and *via* a pinacol-type rearrangement of an intermediate epoxide) and ring-enlargements by pinacol-type rearrangement: these chemical reactions were sometimes accompanied by bioactivation. Extension of this approach (i) to the biological fate, for example of hindered phenolic antioxidants and direct alkylating agents, (ii) to the rearrangement of metabolically generated halogeno-alkene epoxides, and (iii) to the interaction of the reactive metabolites with DNA and with cysteinyl and methionyl peptides suggested that chemical reaction processes contribute more extensively to the metabolism of foreign compounds in mammals than is generally supposed. However, recourse to chemical reactions for processes that occur *in vivo* should be made selectively, and one case in the literature, where such a reaction step is implicit (*v. supra*), ought to be reconsidered.

I should like to express my best thanks to Dr. C. W. Suckling, F.R.S., General Manager of Research and Technology, I.C.I. Limited, London, SW1P 3JF, and to Professor R. J. P. Williams, F.R.S., University of Oxford, for their kindness in reading the manuscript.