BIOCHEMISTRY OF PHENOLIC COMPOUNDS

J. B. HARBORNE

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Edited by

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Preface

Until recently, the majority of naturally occurring phenols were ignored by biochemists, who have generally been more concerned with seeking unity rather than diversity in Nature, and were only seriously investigated by chemists. The structural identification, stereochemistry and biogenesis of the more complex phenols have, in fact, provided organic chemists with some of their most intriguing and intractable problems. In the last decade, however, the emphasis has shifted towards the biochemistry of this chemically diverse group of natural constituents and increasing efforts have been made to determine more precisely their function in plants and animals.

This change of emphasis was due to two notable discoveries. Firstly, Bate-Smith and others developed the paper chromatography of phenols and were able to show that many of the simpler phenols are not the rare substances they were once thought to be but instead are almost universally distributed in the plant kingdom. Secondly, von Euler, Vogt and Gaddum demonstrated that micro-amounts of certain phenols occur in some of the most vital areas of the animal organism amd notably in nerve tissue and in the brain. In addition, the introduction of ^{14}C tracer techniques has provided the means of studying the biosynthesis of phenols, the results of which show that phenols are metabolically active and by no means inert "end products" of cellular metabolism. It is no longer possible, therefore, to ignore the very real biological importance of these substances and the present volume covers, as comprehensively and authoritatively as possible, the many biochemical aspects of phenols.

The term phenol is used here to include all natural substances with a free or masked phenolic function; while emphasis is naturally on polyphenols having a C_{15} flavonoid nucleus, most other phenols, and particularly those having an additional functional nitrogen group, are covered. The chemical background and methods of identifying these substances in biological materials are dealt with in the opening chapters. Their distribution, taxonomic significance, genetics, metabolism, biosynthesis, enzymology and function in both plants and animals are considered, in turn, in subsequent chapters. In all, this book will have fulfilled its purpose if it directs attention anew to the potentially important role of phenolic compounds in living systems.

The editor expresses his gratitude to the contributors for their generous and enthusiastic co-operation in this venture. He also thanks his colleagues, and particularly N. W. Simmonds and Dr. Tony Swain, for their help with editorial problems and Miss Janet Corner, for patiently compiling the indexes. The support and encouragement of the Academic Press is gratefully acknowledged.

JEFFREY B. HARBORNE

Bayfordbury
March, 1964

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CHAPTER 1

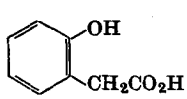
Structure and Reactivity of Phenolic Compounds

R. H. THOMSON

Chemistry Department, University of Aberdeen, Scotland

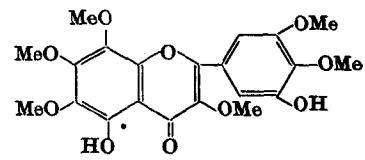
I. Introduction

The expression "phenolic compounds" embraces a wide range of substances which possess an aromatic ring bearing a hydroxyl substituent, including their functional derivatives. Among the natural phenolic compounds, of which several hundreds are known, the flavonoids and their relatives form the largest group but phenolic quinones, lignans, xanthones, depsidones, and other groups, exist in considerable numbers as well as many simple monocyclic phenols. Some recent examples are shown below ((1)–(6)). Several important polymeric



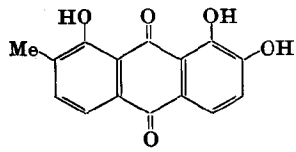
(1)

2-Hydroxyphenylacetic acid (*Astilbe* spp.)



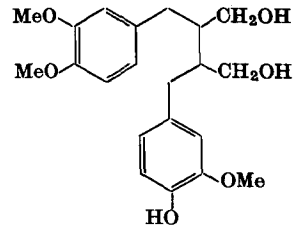
(2)

Digicitrin (*Digitalis purpurea*)



(3)

Cladofulvin
(*Cladosporium fulvum*)



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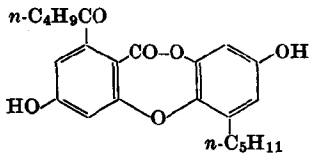
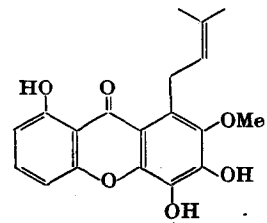
Seco-isolariciresinol
(*Podocarpus spicatus*)



(5)

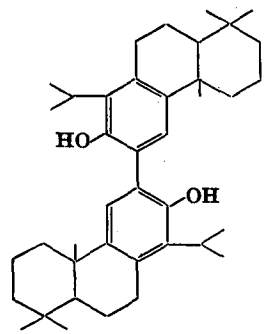
Norlobaridone
(*Parmelia conspersa*)



(6)

Celebixanthone
(*Cratoxylon celebicum*)

materials, lignins, melanins, tannins, are polyphenolic and occasional phenolic units are encountered in peptides and proteins, in macrolides, and amongst alicyclic natural products exemplified by the phenolic

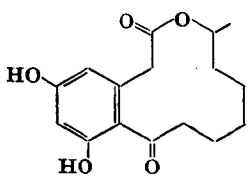


(7)

Podototarin
(*Podocarpus totara*)

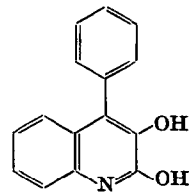
sterols and a number of terpenoid phenols, e.g. (7). Any group of alicyclic compounds containing six-membered carbocyclic rings may be regarded as a potential source of phenols, and the existence in nature of tri- and

tetraterpenoid phenols may be predicted. (Carotenoids with aromatic rings are already known (Yamaguchi, 1958, 1959) and a quinonoid triterpene has been recognized (Grant *et al.*, 1960; Harada *et al.*, 1962.) In addition, a multitude of natural phenolic compounds have been identified in recent years which fall outside the main groups described so far; structures ((8)–(12)) are illustrative.



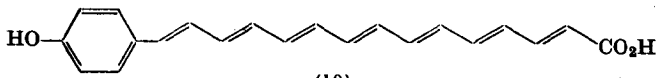
(8)

Curvularin
(*Curvularia* spp.)



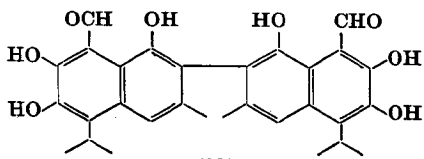
(9)

Viridicatin
(*Penicillium viridicatum*)



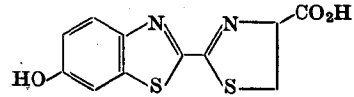
(10)

Cortisalin
(*Corticium salicinum*)



(11)

Gossypol
(*Gossypium* spp.)



(12)

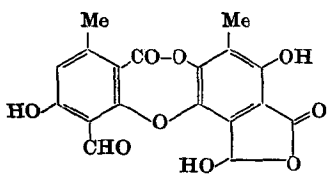
Luciferin
(*Photinus pyralis*)

The seemingly bewildering array of natural phenols forms a more coherent picture when biogenetic considerations, and the chemical reactivity of phenols, are taken into account. The former are discussed in Chapter 8 and it is the purpose of this chapter to consider the properties of phenols with particular respect to those occurring naturally. Most of the fundamental laboratory reactions of phenols occur both *in vivo* and *in vitro* and they can be roughly divided into those which concern the hydroxyl group and those based on the aromatic ring; this division is somewhat arbitrary as reactions may involve both parts of the molecule.

II. Properties and Reactions of the Hydroxyl Group

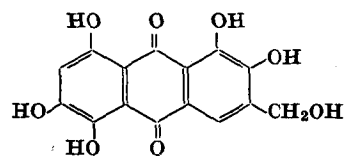
A. Acidity

Phenols are weakly acidic (phenol itself has pK_a 9·98) but there are significant variations arising from the inductive, mesomeric, and occasionally steric effects of substituents. The classical example of a strongly acidic phenol is picric acid (pK_a 0·71) which may be contrasted with pentamethylphenol which is scarcely soluble in hot aqueous potassium hydroxide, and with 2,4,6-tri-t-pentylphenol which does not react with sodium in liquid ammonia nor with sodium–potassium alloy in ether. These are extreme cases and, in the absence of nitro groups, the more acidic natural phenols usually possess carbonyl substituents. Phenols with one carbonyl group are generally soluble in aqueous sodium carbonate and a few (some 7- and 4′-hydroxyflavonols for example (Briggs and Locker, 1951)) are soluble in bicarbonate. If two carbonyl substituents are present, the acidity is increased and such compounds as 7-hydroxy-1,2-naphthoquinone, norstictic acid (13) (Asahina and Shibata, 1954) and certain poly-β-hydroxyanthraquinones (e.g. (14))



(13)

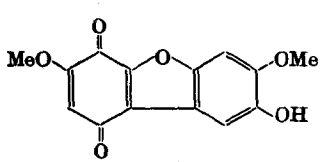
Norstictic acid
(*Lobaria pulmonaria*)



(14)

Asperthecin
(*Aspergillus nidulans*)

(Howard and Raistrick, 1955; Birkinshaw and Gourlay, 1961) dissolve in aqueous sodium bicarbonate. These, however, are the exception rather than the rule, as is the phenolic compound (15) which readily dissolves in cold aqueous sodium carbonate forming a blue solution, the colour being that of the anion of (16) (Shand and Thomson, 1963). Differences in



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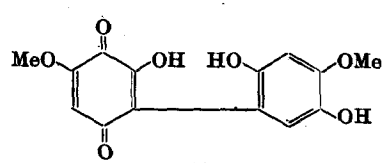


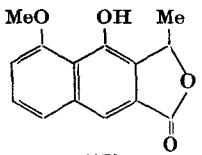
(16)

acidity are of obvious value in the separation of mixtures and are widely exploited, in conjunction with other methods, in the isolation of natural

products. It should not be forgotten that β-di- and triketones, which occur in hops and various essential oils, are distinctly acidic and simulate phenols in this and other ways (e.g. triacetylmethane has pK_a 5·81; cf. (82) and (83)).

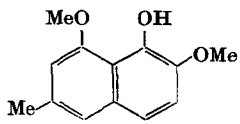
Polyalkylated (and hence weakly acidic) natural phenols are not common but totarol and related diterpenoid phenols (e.g. (7)) are cryptophenolic and appear mainly in the "neutral" fractions during routine extractions (Cambie and Mander, 1962; Cambie *et al.*, 1963).

Some effects of hydrogen bonding are discussed below but it may be noted here that intramolecularly bonded phenols tend to be less readily soluble in alkali than their non-bonded isomers. Thus β- but not α-hydroxyanthraquinones and 7- but not 5-hydroxyflavones are soluble in



(17)

Eleutherol
(*Eleutherine bulbosa*)



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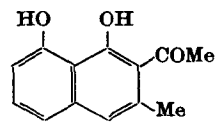


(19)

Musizin
(*Maesopsis eminii*)

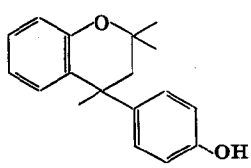
aqueous sodium carbonate, and 5-hydroxyflavones may be insoluble in aqueous sodium hydroxide (Briggs and Locker, 1951). *peri*-Methoxynaphthols (e.g. (17) and (18)) are frequently cryptophenolic dissolving readily in Claisen's alkali (aqueous methanolic potassium hydroxide) but with difficulty, or not at all, in aqueous sodium hydroxide. However, there are irregularities since rubrofusarin (26) monomethyl ether and both monomethyl ethers of musizin (19) are readily soluble in aqueous alkali.

B. Hydrogen Bonding

Unless sterically hindered, all phenols take part in hydrogen bonding. The system O—H···O is the most important and natural examples of both inter- and intramolecular bonding are legion. It is well known that many physical properties are affected by hydrogen bonding (Pimentel and McClellan, 1960); these include vapour pressure, melting point and boiling point, solubility, crystal structure, and ultraviolet, infrared, and nuclear magnetic resonance spectra, some of which are used to advantage in isolation and purification procedures, and for purposes of identification, which need not be elaborated here. In general, intramolecularly bonded compounds are more easily manipulated than intermolecularly

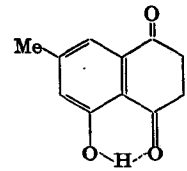
bonded substances, many of which are polymeric in the solid state. Polyphenolic flavonoid and quinonoid compounds, for example, have high melting points and are inconveniently insoluble in the usual solvents so that solution spectra are often unobtainable. Again, simple phenols are water-soluble but this diminishes with increasing molecular complexity and is not offset by an increase in the number of phenolic groups owing to the tendency to form a strong crystal lattice structure with the maximum number of hydrogen bonds. Thus phloroglucinol (m.p. 219°), despite its three phenolic groups, is very much less soluble in water (1·13 g/100 g) than is resorcinol (m.p. 118°) (167 g/100 g), and of course, it is insoluble in non-polar solvents as well.

With regard to the purification of phenols, an interesting disadvantage arising from hydrogen bonding is their pronounced tendency to form flat hexagonal structures composed of six phenolic groups linked together by hydrogen bonds, which in turn may form the ends of large cavities within the crystal, the sides being formed by the aromatic rings



(20)

Dianin's compound



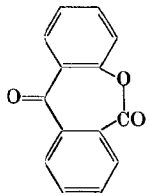
(21)

(*Diospyros ebenum*)

and other parts of the phenol molecule. These molecular cages account for the tendency of phenols to give rise to inclusion compounds, of which the quinol clathrates are the best known. A more striking example is Dianin's compound (20) which forms inclusion compounds with more than fifty organic solvents (Baker *et al.*, 1956), and since phenol itself forms clathrates this tendency to retain solvent molecules is potentially troublesome in the purification of phenols.

Although relatively weak, hydrogen bonds are an important structural feature of many compounds, stabilizing particular isomers or conformations, or directing the course of a particular reaction. An example is seen in the diketone (21); undoubtedly this is stabilized by the intramolecular hydrogen bond which inhibits tautomerization to a trihydroxynaphthalene system. The directive influence of hydrogen bonding on the course of a reaction is shown in the dehydration of phenolic keto-acids of type (23; R = H). In cold acetic anhydride, containing sodium acetate, (23; R = H) rapidly undergoes intramolecular

condensation to form the lactone (22) whereas other dehydrating agents give only the lactide (24) (Baker *et al.*, 1952). The explanation is that in



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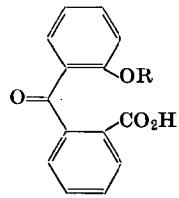

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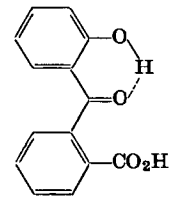
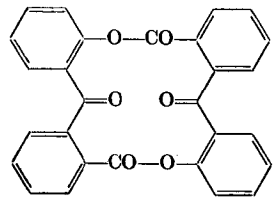


(23a)

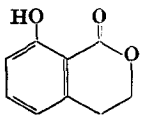


(24)

the former case lactone formation is preceded by acetylation of the phenolic group to give (23; R = Ac) which then undergoes a base catalysed intramolecular trans-esterification, whereas other dehydrating agents leave the phenolic group free and consequently the molecule takes up the hydrogen-bonded configuration (23a). This arrangement, while quite unfavourable for intramolecular condensation, is suitably disposed for intermolecular reaction to form the lactide.

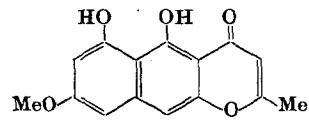
In general the chemical reactivity of phenolic groups is diminished by intramolecular hydrogen bonding and in extreme cases phenolic properties (alkali solubility, formation of ethers, esters, etc.) are almost completely suppressed. Normally, bonding is stronger in *peri*-hydroxycarbonyl compounds (flavones, xanthones, quinones, etc.) than in *o*-hydroxycarbonyl compounds (ketones, esters, etc.), and stronger in *peri*-hydroxynaphthols than in catechols, i.e. six-membered chelate rings are more stable than five-membered rings. This can be seen in methylations using diazomethane (a reaction sensitive to intramolecular bonding), where catechols readily form dimethyl ethers but 1,8-dihydroxynaphthalenes yield only monomethyl ethers (the phenol (18), for example, does not react with diazomethane). Neither *peri*- nor *o*-hydroxycarbonyl compounds are methylated by diazomethane as a rule but the difficulty can be overcome in some cases (e.g. (25)) by using methanol as solvent, a large excess of reagent, and prolonged reaction time, or by methylation with methyl sulphate or methyl iodide. Where

methylation is unexpectedly facile, the phenolic group usually lies in a crowded position such that one or more substituents may be forced out of the plane of the aromatic ring with consequent weakening of the hydrogen bond. Transmission of steric effects through a series of adjacent



(25)

Mellein
(*Aspergillus melleus*)

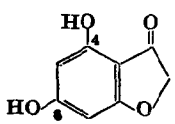


(26)

Rubrofusarin
(*Fusarium* spp.)

groups is well known (Hunter, 1954), and is further exemplified by cladofulvin (3) which yields the 1,2-dimethyl ether on treatment with diazomethane (Agosti *et al.*, 1962), and rubrofusarin (26) in which the hydroxyl group *ortho* to the carbonyl group is preferentially methylated (Bycroft *et al.*, 1962). On the other hand, a number of simple ketoquinols (e.g. 2,5-dihydroxyacetophenone) are inert to diazomethane and do not even form a monomethyl ether (Farmer *et al.*, 1956).

In addition to the size of the chelate ring, the size of the ring containing the carbonyl group also affects bond strength. If the carbonyl group is located in a five-membered ring the O—H···O distance is increased to *ca.* 3 Å, the hydrogen bond is consequently weak and the phenolic group exhibits normal properties. This may be illustrated by comparison of the coumaranone (27) and the chromanone (28). Treatment of the latter with



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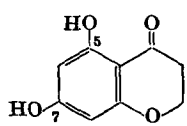
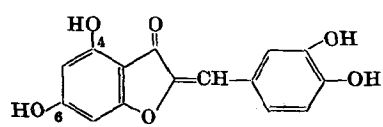


(28)

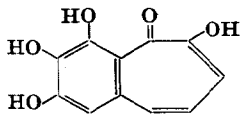


(29)

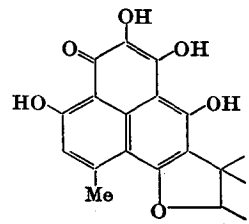
Aureusidin
(*Antirrhinum majus*)

diazomethane yields the 7-monomethyl ether as expected, whereas the coumaranone gives the 4-monomethyl ether (and then the 4,6-dimethyl ether). Preferential methylation of the 4-hydroxyl group in (27) may reasonably be attributed to its greater acidity but the essential difference between the homologues (27) and (28) lies in the strength of the intramolecular hydrogen bond. This is borne out by infrared data (Farmer *et al.*, 1956). Consequently aurones hydroxylated at position 4 (e.g. (29))

behave normally towards diazomethane. In *peri*-hydroxycarbonyl compounds in which the carbonyl group is part of a seven-membered ring, the intramolecular bond is strong. This is evident from physical properties and failure to react with diazomethane; purpurogallin (30), for example, forms the 2′,3′,4-trimethyl ether with this reagent.



(30)
Purpurogallin



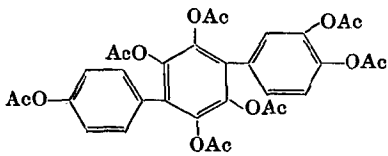
(31)
Atrovenetin
(*Penicillium atrovenetum*)

Intramolecularly-bonded phenolic groups normally show only slight resistance to acylation reactions. In the absence of catalysts (e.g. acetylation with acetic anhydride alone) they may remain inert at moderate temperatures; in the presence of catalysts (e.g. sodium acetate), there is rarely any difficulty and nearly all phenolic groups can be acylated with ease. A notable exception is 9-hydroxyperinaphthen-1-one which has resisted all attempts at alkylation and acylation (acetylation with ketene does not appear to have been tried); however, the 2,3-dihydro derivative readily forms an acetate (Loudon and Razdan, 1954; Koelsch and Anthes, 1941). Similarly the natural derivative, atrovenetin (31), forms only a triacetate, although two isomeric tetramethyl ethers can be obtained by further methylation of the trimethyl ethers with methyl iodide–silver oxide. Both trimethyl ethers are insoluble in aqueous sodium hydroxide and it is of interest that the yellow isomer, which is distinctly basic, forms an acetate perchlorate (Neill and Raistrick, 1957; Paul *et al.*, 1962). In this reaction, protonation of the carbonyl group weakens the hydrogen bond and permits normal acylation to occur. This probably explains why intramolecularly bonded phenolic groups are readily esterified as a rule in the presence of an acid catalyst.

C. Esterification

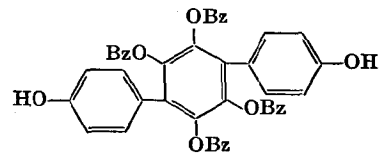
Although phenolic groups are normally esterified *in vitro* without difficulty, except in a few cases where exceptionally strong hydrogen bonding or steric hindrance reduces them to the status of tertiary

alcohols, natural aryl esters are uncommon. Apart from depsides and gallotannins, there are only the rare fungal metabolites (32) and (33). The depside linkage appears to be derived only from gallic and orsellinic acids, and their close relatives, and the numerous other phenolic acids,



(32)

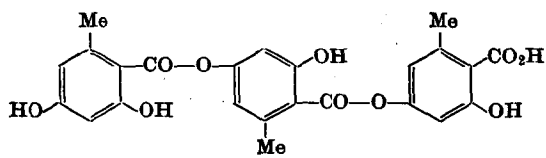
(Ac = CH_3CO–)
Protoleucomelone
(*Polyporus leucomelas*)



(33)

(Bz = PhCO–)
Aurantiacin leucodibenzoate
(*Hydnum aurantiacum*)

notably the very widely distributed hydroxycinnamic acids, do not appear to undergo intermolecular condensation although their aliphatic esters (e.g. with quinic acid) occur frequently. The mechanism of depside biosynthesis, which involves selective esterification of hydroxyl groups in polyphenolic acids, is not known; in (34) the most accessible hydroxyl



(34)

Gyrophoric acid
(*Gyrophora pustulata*)

groups are acylated but this is not always the case. Laboratory syntheses necessitate protection of appropriate phenolic groups before esterification, followed by removal of the protecting group (Haslam *et al.*, 1961).

o-Hydroxycinnamic acids (in the *cis* form) undergo intramolecular condensation to give lactones (coumarins) which are common in flowering plants. Whereas laboratory methods for the preparation of coumarins start from phenols, in biosynthesis (e.g. in *Melilotus alba*) the phenolic oxygen is introduced at a late stage by enzymic hydroxylation of a *trans*-cinnamic acid to form an *o*-coumaric acid which is subsequently converted to the *cis* acid, and lactonized. There is a need for an efficient laboratory method for the synthesis of phenols by direct hydroxylation. Udenfriend's "model peroxidase system" (EDTA–Fe^{2+}–O_2 (or H_2O_2)–