Proc. Indian Acad. Sci. (Chem. Sci.), Vol. 93, No. 7, September 1984, pp. 1159–1169.

Biosynthesis of 9-phenylphenalenones in plants of the family Haemodoraceae: possibly biomimetic synthesis of lachnanthocarpone by an intramolecular Diels-Alder reaction

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Abstract. All species of higher plants of the family *Haemodoraceae* which have been available for study so far contain pigments derived from 9-phenylphenalenone a type of compound not found in any other living organism. Study of the highly unusual biosynthesis—possibly related to that of the fairly numerous plant constituents related to 1,7-diarylheptane—suggested a convenient, possibly biomimetic total synthesis of lachnanthocarpone, one of the pigments from the haemodoraceous *Lachnanthes tinctoria*. This synthesis involves an intramolecular Diels-Alder reaction of an intermediate 1,7-diarylheptanoid very closely related to a natural compound of this class.

Keywords. 9-phenylphenalenones; pigments of haemodoraceous plants; biomimetic synthesis; intramolecular Diels-Alder reaction; natural 1,7-diarylheptanoids.

The Haemodoraceae constitute a small family (~ 17 genera, many of them monotypic) of monocotyledonous plants closely related to the better known Amaryllidaceae; so closely, as a matter of fact, that the genus *Lophiola* (one species growing in Newfoundland and in the so-called Pine Barrens near the coast of New Jersey, USA) has been assigned at different times to either family; recent evidence favors its inclusion among the Amaryllidaceae. Plants of the family Haemodoraceae occur mostly in the Southern hemisphere: Australia, Tasmania, South Africa, South America; the genus *Xiphidium* (one species) inhabits the Caribbean region and another one, the monotypic *Lachnanthes* (formerly *Gyrotheca*) occurs in swampy areas near the Atlantic coast of the United States as far north as Massachusetts. No species seems to occur in India.

Many, although not all, species of the Haemodoraceae have strikingly colorful root systems: blood-red tubers in *Haemodorum*, short orange roots and long runners with an orange core surrounded by a thin magenta layer in *Lachnanthes* ('red root' or 'paint-root'). Also the name of one of the still uninvestigated genera, *Pyrrorhiza*, means 'fire-red root.'

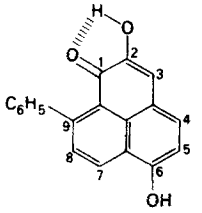
Most of the genera of the family are very small (1–5 species); only the type genus *Haemodorum* of Australia and Tasmania (~ 20 species), *Anigozanthos*, and *Conostylis* are more numerous. *Haemodorum* was the first one to be the subject of detailed chemical study, since 1955, by Prof. R G Cooke and his coworkers in Melbourne, Australia. Subsequently, the chemistry of the constituents of the North American *Lachnanthes tinctoria* was examined by the author and Dr J M Edwards at the National Institutes of Health; this study was continued by Dr Edwards at the University of Connecticut, and expanded to a scrutiny of the biosynthesis. Work on additional genera has been carried out by both groups; the research of Cooke *et al* on *Anigozanthos* and related genera deserves special mention.

Haemodoraceous plants are of particular interest to bioorganic chemists, biochemists, and chemotaxonomists for several reasons:

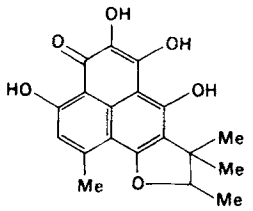
(1) They constitute an instance of a strict 1:1 relationship between phytochemistry and taxonomy: all species which have been available so far for chemical study were found to contain pigments derived from 9-phenylphenalenone,* a class of compound which has never so far been encountered in any other organism. While such a precise correspondence is not unique—*cf.* the exclusive replacement of anthocyanins by the betacyanins in the Centrosperms, a group of about eight closely related plant families—it is decidedly uncommon. Pigments derived from phenalenone—*not* from 9-phenylphenalenone, though—are found in certain molds. Comparison of the structure of lachnanthocarpone from the haemodoraceous *Lachnanthes tinctoria* with that of the mold phenalenone atrovenetin shows the differences in carbon skeleton and location of oxygenated substituents. A comprehensive review of both classes of phenalenones has been published recently (Cooke and Edwards 1981); it should be consulted for more detailed information, and for literature not specifically quoted here.

(2) The 9-phenylphenalenones from haemodoraceous plants must obviously arise through a very unusual biosynthetic pathway, while the mold phenalenones clearly show the 1,3-distribution of oxygenated substituents characteristic of polyketides, with the additional hydroxyl in position 2, and a mevalonate-derived C_5 unit attached. In contrast, the pattern of these substituents in lachnanthocarpone and its congeners precludes a polyketide origin and is more consistent with a derivation from the shikimate pathway. This is borne out further by the occurrence of certain compounds of this group, to be shown later on (scheme 3, below) in which oxygenated substituents occur on the isolated aromatic ring in the positions 4; 3,4; 3,4,5 with respect to C-9 of the phenalenone system. This distribution is highly diagnostic of shikimate-derived substances, and supports such an origin at least for this part of the molecule. On the other hand, the C_{19} skeleton of the 9-phenylphenalenone system can not be derived in a simple fashion from the shikimate pathway with its strong prevalence of C_6–C_3 units allied to phenylalanine, tyrosine, and cinnamic acid. Discussion of the hypotheses formulated for the actual biosynthetic origin of the 9-phenylphenalenones, of the available experimental evidence bearing on this question, and of a synthesis of one such pigment suggested by them, will form the main subject of this paper.

(3) Biological action of certain haemodoraceous plants or their constituents has been observed (Cooke and Edwards 1981). *Haemodorum corymbosum* is stated to be



Lachnanthocarpone



Atrovenetin

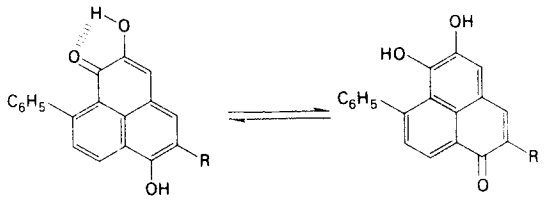
* Absence (Edwards *et al* 1970) of such pigments from *Lophiola americana* constitutes one of the arguments for placing this species in the Amaryllidaceae rather than the Haemodoraceae.

poisonous to animals; its main phenalenone pigment, the cellobioside haemocorin, has antitumour and antibacterial action.

At least one species, the North-American *Lachnanthes*, has been shown to have still another biological activity. The earliest indication of this property is found in an illustrious source: Darwin, in his 'Origin of Species,' quotes a letter from a correspondent, who reports that pigs eat this plant, and that black animals can do so with impunity, while white ones suffer poisoning, which stains the bones pink and causes the hooves to drop off. This sensitivity of light-colored animals, and the resistance of dark ones, was tentatively interpreted later on* as a photosensitization phenomenon (so-called photodynamic effect), *i.e.* oxidative destruction of biological material, often by singlet oxygen formed photochemically under the influence of a sensitizer†. No evidence for this interpretation of the effect of *Lachnanthes* was available at that time. At the suggestion of the author, Kornfeld and Edwards in 1972 examined extracts from various parts of the plant for photodynamic action against *Staphylococcus epidermidis* which, as a gram-positive organism, is quite sensitive to such action. Marked activity was found in extracts from the seed-pods, and in the aglycones of the glycosidic root pigments.

Chemistry of the pigments from Haemodoraceous plants. Only a brief review of the salient aspects of the chemistry of these 9-phenylphenalenones and their allies can be given here, with special attention to those features which are significant for the discussion of problems of biosynthesis. For complete information see the recent review by Cooke and Edwards (1981).

One of the significant properties of most of these pigments is the general occurrence of tautomerism, of the kind shown below, in 6-hydroxy-1-phenalenones, a class which includes the majority of the known pigments from the Haemodoraceae.



R = H, OH, or OMe

This tautomerism leads to the formation of two series of derivatives on methylation and esterification, a circumstance which was helpful during the initial work of Cooke on the structure of haemocorin, the first of these pigments to be elucidated (see below), but which becomes a distinct nuisance in isotope work, where derivatization of the scarce labelled material results in appearance of two compounds instead of one.

Another general property of these substances, very useful in research on structure and biosynthesis, is their behaviour on oxidation by a variety of techniques, when an

* Blum 1964; photodynamic action of a variety of pasture plants causes serious problems with raising of livestock in many parts of the world, *Hypericum* spp. (St. John's Wort) being the best known culprits.

† It was a review article on this effect which brought *Lachnanthes* to the author's attention in about 1924 and stimulated the desire to examine this plant. After an unusually long incubation period, actual work on *Lachnanthes* became possible in 1968.

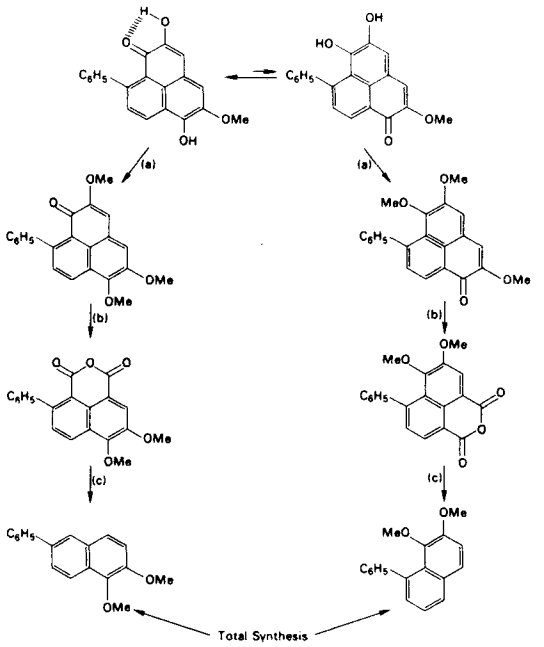
oxygenated carbon adjacent to the carbonyl group is eliminated quite specifically, naphthalic anhydrides being formed. This reaction also proceeds on aerobic irradiation (Narasimhachari *et al* 1968); as will be shown below, it is not the only photochemical transformation which ketones of the phenalenone series can undergo.

Both tautomerism and specific oxidative removal of an oxygenated carbon next to a carbonyl are demonstrated in scheme 1, which shows the main reactions used by Cooke *et al* (1958) in their elucidation of the structure of haemocorin aglycone, the first natural compound which was found to be a 9-phenylphenalenone.

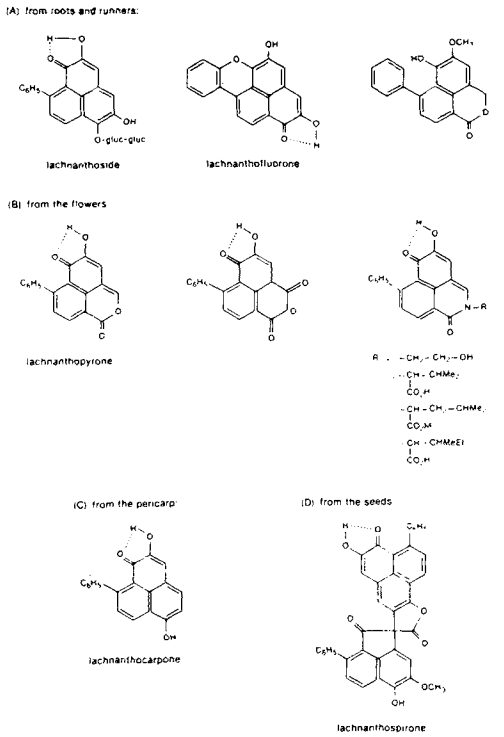
The characteristic oxidative elimination of the carbon next to a keto group may well take place *in vivo* as well as *in vitro*; both groups of naturally-occurring phenalenones, those from molds as well as those from haemodoraceous plants, are frequently accompanied by compounds carrying oxygen (in a few cases nitrogen) in place of one of these carbons. It is reasonable to assume that these heterocycles are formed from intact phenalenones by processes similar to the ones observed *in vitro*; however, this hypothesis has apparently not been tested experimentally.

The components of *L. tinctoria* identified so far are shown in scheme 2, which will exemplify the occurrence of 9-phenylphenalenones and of the related heterocycles presumably derived from them.

A few features of this scheme require comment. Intact phenalenones occur in the roots of *L. tinctoria*, in the pericarp (the brown pulp surrounding the seeds in the fruit capsules) and, in the form of the dimer lachnanthospirone, also in the seeds. In the



Scheme 1.



Scheme 2.

roots, the phenalenones are accompanied by several oxygen heterocycles which are naphthalides.

In contrast to the occurrence of the compounds with the intact carbon skeleton in these parts of the plants, such pigments seem to be lacking in the flowers, which so far have yielded only O- and N-heterocycles.

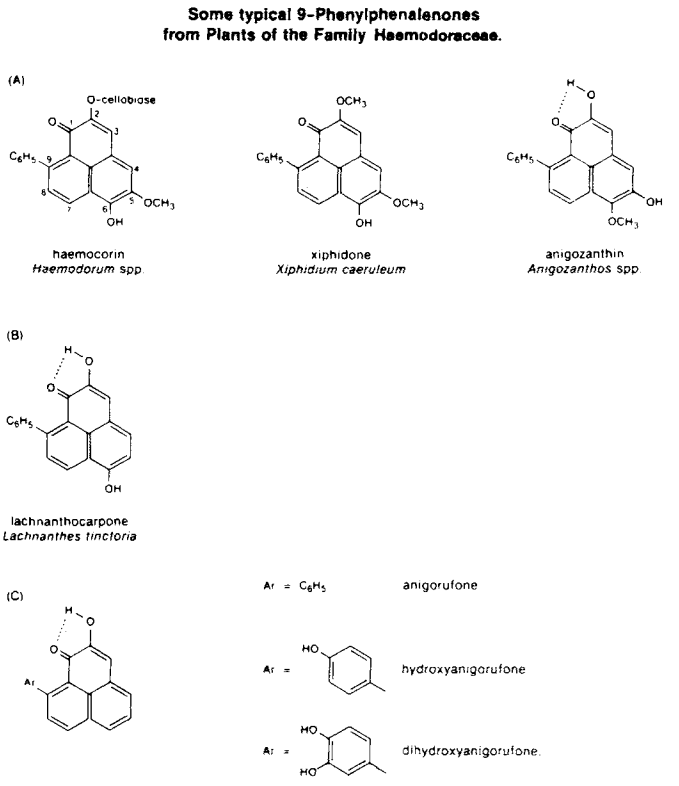
The biosynthesis of the acenaphthenone moiety of lachnanthospirone presents an unusual problem, which has not yet been approached experimentally. The structure of this moiety suggests that it may be formed from a 1,2,3-triketo-dihydrophenalene by some biochemical equivalent of a benzilic acid rearrangement, with concomitant coupling of the resulting entity with an intact phenalenone unit by phenol dehydrogenation. Benzilic acid rearrangements of the type assumed here are known *in vitro*; *cf.* the conversion of dihydrophenalene-1,2,3-trione into acenaphthenequinone (Rubin 1975).

The structure of lachnanthofluorone given in scheme 2 may require revision. The nature of the parent 1H-naphtho[2,1,8-*mna*]xanthen-1-one system present in this red to mauve, intensely red-fluorescing pigment is established beyond reasonable doubt. However, the hydroxyl placed at C-5 in the formula may actually occupy a different position (Cooke and Edwards 1981). (The strongly polar nature of the pigment, and its occurrence in very small amounts only, made its investigation quite difficult). Compounds derived from the same ring system are formed on brief irradiation of 9-phenylphenalene-1-ones (Weiss and Edwards 1969), sometimes in fairly high yield (Cooke and Dagley 1979). Two more pigments of this type, the haemofluorones A and

B, have recently been isolated from several genera of the Haemodoraceae by Cooke *et al* (Cooke and Edwards 1981; Cooke and Dagley 1979).

Again, formation, presumably photochemical, of the naphthoxanthenones in the plant from 9-phenylphenalenones is a reasonable but unproven hypothesis. If correct, it would constitute an interesting example of a photochemical step in the biosynthesis of a secondary metabolite*. One hint in this direction may be furnished by the runners of *L. tinctoria* which, as mentioned before, have a magenta outer layer and an orange core, the main pigment of which is the orange glycoside lachnanthoside (see scheme 2). It seems at least possible that the magenta color may be caused by naphthoxanthenones formed photochemically from the phenalenones of the core. However, the chemical nature of the magenta pigments has not yet been investigated separately.

In scheme 3, representative examples of 9-phenylphenalenones from haemodoraceous plants have been assembled. No complete coverage is intended (for a complete listing of these pigments and their congeners, see Cooke and Edwards 1981), but the structures shown should suffice to bring out a striking regularity in the patterns of oxygenated substituents, a pattern which would not be altered by inclusion of



Scheme 3.

* For a discussion of the possible involvement of photochemical reactions in biosynthetic sequences, see Weiss and Edwards (1980).

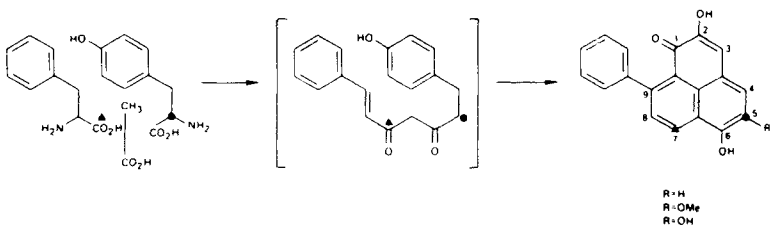
additional examples. It is apparent that these pigments can carry 2, 3, or 4 oxygenated functions on the phenalenone system; furthermore, such functions are invariably present in positions 1 and 2, and often but not always in positions 6, or 5 and 6. They never occur elsewhere*; in particular, no case of oxygenation at C-7 has been observed so far, although, as will be shown later, this carbon is derived from a carboxyl. It can hardly be doubted that this pattern of hydroxylation in some way reflects the mode of biosynthesis of the phenalenone system.

Scheme 3 also exemplifies the occurrence and distribution of the oxygenated functions on the aromatic ring attached at C-9, which have been mentioned before. Other instances of such substituents occur among the naphthoxanthenones (Cooke and Edwards 1981; Cooke and Dagley 1979) and the naphthalides, of which one example (with unsubstituted-C_6H_5) is shown in the top row of scheme 2.

Biosynthesis of the 9-phenylphenalenones It has been mentioned already that the phenalenones from haemodoraceous plants show none of the structural characteristics of polyketides, and that the aromatic ring at C-9 should, from the pattern of those oxygenated substituents which are present in some instances, be derived from shikimate, while it is not possible to construct the entire C_{19} skeleton from shikimate in a reasonable way.

An attractive scheme has been proposed by Thomas in 1961. It postulates an origin of the C_{19} skeleton through condensation of one molecule each of phenylalanine and tyrosine (or their metabolic equivalents) with one molecule of acetic acid, with loss of one of the three carboxyls, to give a C_{19} intermediate derived from 1,7-diarylheptane, which then cyclizes to the 9-phenylphenalenone (scheme 4). This scheme was supported by the incorporation of all three components into the aglycone of haemocorin (*cf.* scheme 1) in *Haemodorum corymbosum* (Thomas 1971, 1973), and into that of lachnanthoside in *L. tinctoria* (Edwards *et al* 1972), and especially by the demonstration (Thomas 1971, 1973) that radioactivity from 2-^{14}tyrosine is exclusively localized at C-5, the expected position†; oxidative removal of this carbon leads to complete loss of label.

Studies of this biosynthesis in *L. tinctoria* showed that the carboxyls from both 1-^{14}C-labelled phenylalanine and tyrosine are efficiently incorporated (the one from phenylalanine quite remarkably so) (Edwards *et al* 1972); it is thus the acetate carboxyl which appears to be lost. However, this carboxyl, too, is incorporated, although to a



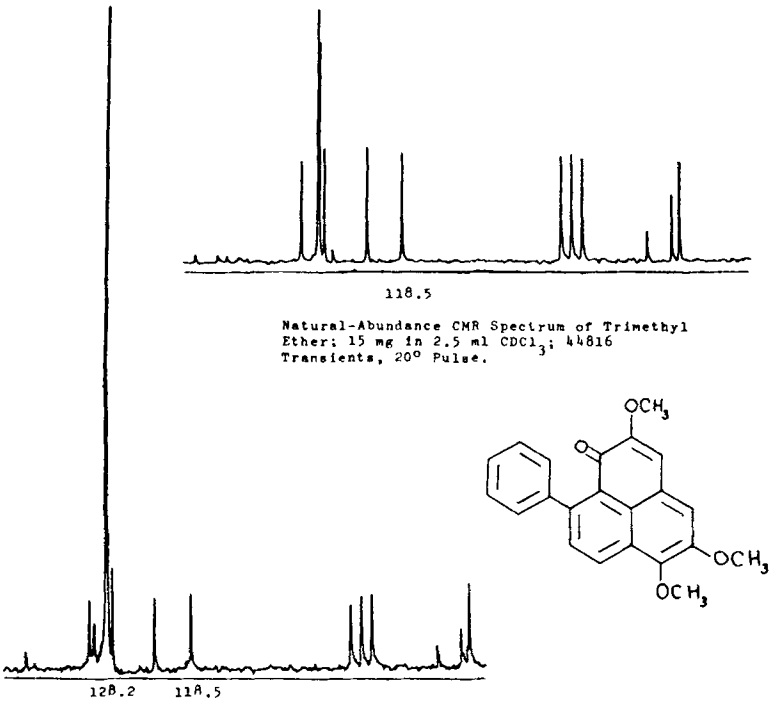
Scheme 4.

* Oxygenated substituents on the isolated aromatic ring at C-9 are disregarded here; these regularities do *not* seem to hold for the naphthoxanthenones (Cooke and Edwards 1981).

† In principle, the C_{19} skeleton could also originate from phenylalanine, tyrosine, and a C_1 unit. However, formate was poorly incorporated into haemocorin aglycone (Thomas 1971, 1973).

much smaller extent than the methyl, and this incorporation of CO_2H is low in experiments where the biosynthesis was allowed to proceed only for the shortest time compatible with reliable measurement of the radioactivity (1 day); it is much higher in experiments extending over three days. It seems therefore that C-1 of acetate is indeed eliminated during the biosynthesis, but that part of the resulting CO_2 is re-absorbed and recycled.

It would of course be highly desirable to determine the localization of the activities from the variously labelled precursors by chemical or spectroscopic techniques. However, the difficulties of a specific chemical degradation of such compact ring systems on a micro-scale are notorious, and no feasible method seems available, e.g., for isolating C-6a which, on the basis of scheme 4, should be the carbon derived from C-2 of acetate. Fortunately, the incorporation of the carbonyl of phenylalanine is high enough for ^{13}C-NMR to be applicable. The allocation of all observed peaks to the 19 individual carbon atoms presents a formidable problem which has not yet been completely solved; all carbon atoms except the carbonyl are aromatic or vinylic; the signals from the six quaternary carbons are closely crowded together, and the same is true for those from the nine carbons which carry a hydrogen atom. However, among the latter, the signal of C-7 of the lachnanthoside aglycone trimethyl ether (=dimethyl ether of haemocorin aglycone) can be identified by proton decoupling, since the proton on C-7 yields the most deshielded resonance in the ^{1}H-NMR spectrum. Feeding of 1-^{13}C-phenylalanine (Harmon *et al* 1977) led to a huge increase of just this signal, showing that incorporation has occurred in precisely the place where it was expected on the basis of Thomas' hypothesis.



While these results do not constitute conclusive proof for the correctness of scheme 4, they are evidently compatible with it, and provide substantial support.

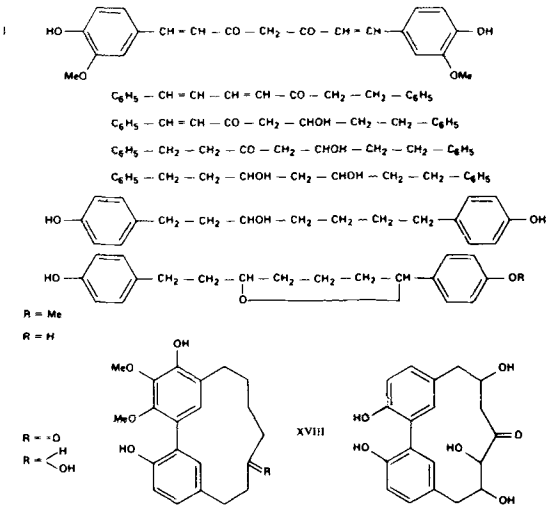
1,7-diarylheptanoids In formulating his hypothesis, Thomas (1961) has pointed out the close structural analogy between his postulated Ar-C_7-Ar intermediate (see scheme 4) and the long-known compound curcumin, the main pigment of turmeric (*Curcuma longa*, Zingiberaceae), which was at that time the only diarylheptanoid compound known to occur in nature. Its structure is shown in the top line of scheme 5.

Some naturally-occurring diarylheptanoids In recent years, many additional compounds with this carbon skeleton have been discovered; the 1,7-diarylheptanoids now form a sizeable class of natural compounds. Representatives of this class have been isolated from several quite unrelated plants, which include taxa from both monocotyledonous (Zingiberaceae) and dicotyledonous families (Betulaceae, Compositae, Myricaceae, Leguminosae). Representative structures of such substances are given in scheme 5, where the second compound shown should be noted for later reference. This dienone, 1,7-diphenyl-1,2-heptadien-5-one, has been isolated by Suga *et al* (1971) from an alder, *Alnus pendula* (Betulaceae); it has been synthesized by straightforward methods (Sakakibara 1972).

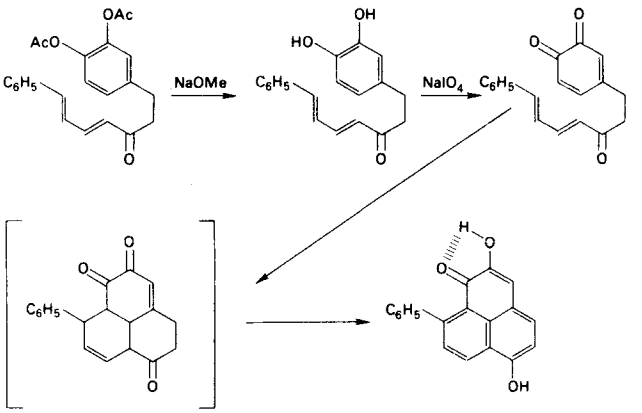
No 1,7-diarylheptanoid has been isolated so far from a haemodoraceous plant; perhaps merely because attention has been focussed upon their pigmented constituents (Cooke and Edwards 1981).

Except for some research on curcumin (Roughley and Whiting 1973), the biosynthesis of the 1,7-diarylheptanoids has apparently not been studied.

Occurrence of a fair number of natural compounds with the carbon skeleton of the postulated intermediate in the biosynthetic scheme proposed by Thomas may be interpreted as lending further support to this scheme. Assuming that it is fundamentally correct, i.e., that the biosynthesis of the 9-phenylphenalenones does indeed proceed via a 1,7-diarylheptanoid, it becomes pertinent to consider ways in which a suitably



Scheme 5.



Scheme 6.

functionalized compound of this type could cyclize to give a 9-phenylphenalenone. One attractive hypothesis, shown in scheme 6, assumes an intramolecular Diels-Alder reaction of a compound having a 1,3-diene at one end of the straight chain, and an orthoquinonoid ring as the dienophile at the other end. Diels-Alder reaction of such a substance should produce the required tricyclic carbon skeleton; subsequent dehydrogenation would then give the 9-phenylphenalenone. The sequence is exemplified in scheme 6 for the case of lachnanthocarpone. It is an attractive feature of the sequence shown in this scheme that the intermediate orthoquinonoid 1,7-diarylheptadienone is very similar to the naturally-occurring 1,7-diphenylhepta-1,3-dien-5-one (see scheme 5), from which it differs only by replacement of one phenyl group by the orthoquinone ring.

Possibly biomimetic synthesis of lachnanthocarpone Subsequent experimental work by Bazan in Dr Edwards' laboratory showed that the reaction of scheme 6 is entirely feasible (Bazan *et al* 1977, 1978). The diacetate of the starting material shown in the scheme was prepared by a modification of the synthesis (Sakakibara *et al* 1972) of the natural phenyl analog. Saponification with a catalytic amount of NaOMe gave the free ortho-diphenol. This was oxidized with aqueous $NaIO_4$ to the orthoquinone, which was not isolated but extracted into chloroform, and the extract was allowed to stand overnight. Unexpectedly, this technique resulted in the direct formation of lachnanthocarpone in acceptable yield (37%). The assumed primary hydroaromatic Diels-Alder product (scheme 6) was not observed, having evidently undergone some spontaneous dehydrogenation (or perhaps dismutation). It should be feasible to prepare other constituents of haemodoraceous plants by modifications of the sequence.

The synthesis may, or may not, parallel the actual biosynthesis of lachnanthocarpone. One argument for its essentially biomimetic nature is the ease with which it can account for the distribution of oxygenated functions in the 9-phenylphenalenones, which has been discussed before; carbons 1 and 2 *always* carry oxygen—of course, since they come from the orthoquinone grouping which is vital as the dienophile in the Diels-Alder reaction; carbons 6 or 5 and 6 do so frequently but not always, a fact in keeping with the origin of C-6 from a carboxyl in Thomas' scheme; however, the resulting carbonyl at the carbon of the 1,7-diarylheptanoid precursor which corresponds to C-6

of the phenalenone does not participate in the cyclization and may be absent, as it should be in the biosynthesis of the anigorufones which are shown in scheme 3; finally, the strange-appearing absence of any oxygen-containing function at C-7, in spite of its securely established derivation from the carboxyl of phenylalanine (Harmon *et al* 1977), is easily explained by the fact that this oxygen would be lost during the elaboration of the diene system.

It seems that all available evidence suggests—but does not prove—that the 9-phenylphenalenones are indeed formed by a sequence not much different from the one proposed by Thomas (1961, 1971, 1973); that 1,7-diarylheptanoids may well be intermediates; and that their cyclization may occur through some Diels-Alder reaction akin to the one occurring in the synthesis of lachnanthocarpone.

If so, it seems reasonable to assume that the entire group of natural 1,7-diarylheptanoids might be formed in the way proposed by Thomas, whose scheme would then have validity well beyond the small group of the 9-phenylphenalenones for which it was originally formulated. Possibly, only plants of the family Haemodoraceae may have evolved the ability of producing 1,7-diarylheptanoids which contain both diene and dienophile groupings and are thus capable of an intramolecular cyclization to phenylphenalenones.

Besides the 9-phenylphenalenones, quite a few other compounds isolated from natural sources (higher plants and microorganisms) have structures which suggest a formation by a Diels-Alder reaction. The problem of the possible biosynthetic role of such reactions has been discussed in Bazan *et al* (1978).

Dedication This paper is dedicated to Professor P K Bhattacharyya on the occasion of his retirement with respect, affectionate friendship, and all good wishes for many happy, active, and productive years.

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