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Review Article

Naturally Occurring Coumarins and Related Physiological Activities

By TAITO O. SOINE

SPÄTH (1), DEAN (2, 3), and Reppel (4) have written comprehensive reviews pertaining to the chemistry of the naturally occurring coumarins, the latest being that of Dean (3) in 1963. Mention should be made of the compilation by Karrer (5) in 1958 wherein he lists the coumarins that had been isolated from natural sources up to the date of publication, together with references to their isolation, structure elucidation, and synthesis. Most of the authors have commented briefly on the physiological and pharmacological activities associated with this group of compounds as well. The survey of von Werder (6) in 1936 concerning the coumarins as therapeutic agents should be mentioned also in this respect. Further, Bose (7) has summarized most of the biochemical properties of the natural coumarins. Nevertheless, a perusal of the literature indicates that no recent review of the physiological activities of coumarins has been forthcoming; therefore, the present writing attempts to bring together some of the interesting work in this connection.

Coumarins were discovered originally in plants; the name of the group derives from "coumarona," the common name of *Coumarouna odorata*, from which coumarin itself was isolated first by Vogel in 1820. For many years naturally occurring coumarins were the only ones known, and it was not until the synthetic methods of Perkin and von Pechmann in the latter part of the 19th century combined to produce virtually any synthetic coumarin desired. In the words of Späth, the synthetic output of coumarin derivatives of almost every imaginable type was limited only by "die Phantasie und die experimentellen Moglichkeiten der Chemiker." However, it is not the purpose of the present writing to cover the vast literature pertaining to synthetic coumarins, except as it impinges on the basic premise of the review—namely, the physiological activity of naturally occurring coumarins. In this sense, those synthetic derivatives that bear upon the definition of the structure-activity profile of the natural prototype will be considered. It is obvious, as in many other fields, that the natural prototype may not be the best representative exhibiting a given physiologic activity. Nonetheless, the natural product serves a useful purpose in directing further synthetic effort and, on occasion, withstands the competition of its synthetic congeners (*e.g.*, coumestrol and psoralen). It is quite safe to say, however, that all medicinally useful coumarins have had their origins in natural products.

Natural Occurrence and Biosynthesis.—Coumarins occur in all plant parts from roots to flowers and fruits and are found widely spread in various plant families. Späth (1) and Reppel (4) have listed the plant sources and the parts of plants associated with coumarins. In general, they

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are found especially in the grasses (*Poae*), orchids (*Orchidaceae*), legumes (*Leguminosae*), umbellifers (*Umbelliferae*), *Rutaceae*, *Labiatae*, and other families of lesser importance. They are rarely, if ever, found in the conifers, cacti, lilies, or primrose. More recently, a few complex coumarins have been found as metabolic products of bacteria and fungi, the most recent being the aflatoxins which are the result of *Aspergillus flavus* infection of oil-seed cake derived from peanuts (8). Only a few coumarins have been obtained from animal sources, the outstanding ones being the two yellow pigments isolated by Lederer (9) from castoreum. The simple hydroxy and methoxy compounds, as well as coumarin itself, occur widely in many different families, but as the complexity of the compounds increases they seem to be more and more restricted with respect to familial occurrence. Thus, coumarin, umbelliferone, scopoletin, and esculetin occur extensively in many families; but the more complex psoralen, nodakenin, xanthyletin, and calophyllolide occur in only one or two families. However, in the latter case, the isolations have not been confined always to a single genus or species.

A complete consideration of the biosynthesis of

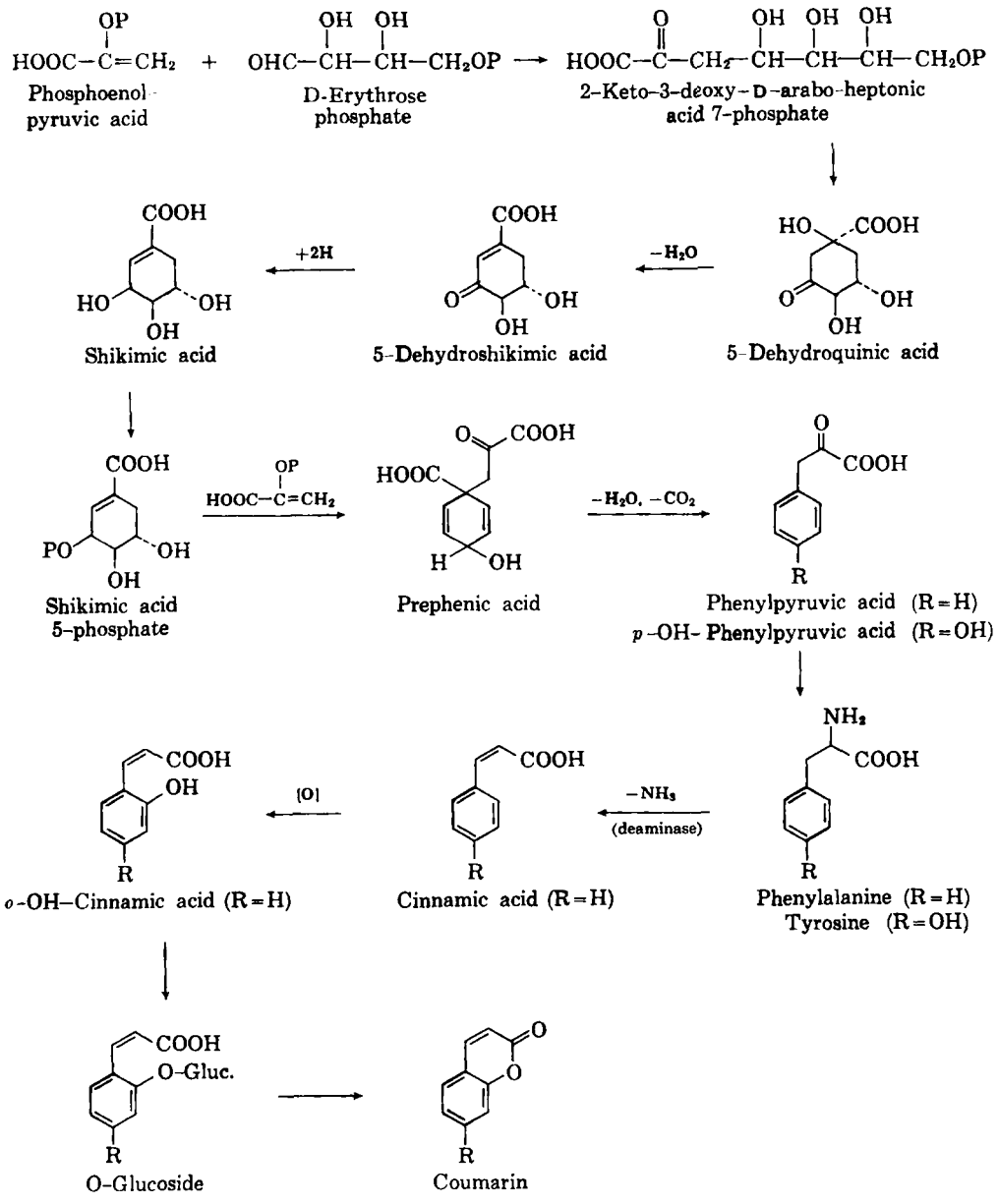


Fig. 1.—Shikimic acid pathway to coumarins.
Key: P = phosphate, R = H or OH as indicated, and Gluc. = glucosyl.

coumarins is somewhat beyond the scope of this review, but it may be pointed out that this field of interest is still in its infancy and much can be expected in the future. Being phenylpropanoid compounds, it has been of considerable interest to researchers to determine which of the two known (10) major pathways is involved in coumarin biosynthesis. These are the Birch-Donovan acetate pathway and the shikimic acid pathway. The acetate route involves head-to-tail condensation of acetate units to build up hydroxylated aromatic rings and has been shown *not* to be the major pathway to coumarins, although coumestrol appears to be an exception, being obtained by way of the isoflavone route (11). On the other hand, the shikimic acid pathway (Fig. 1) has been demonstrated as definitely involved. The initial step is a condensation of phosphoenolpyruvic acid with D-erythrose phosphate to form 2-keto-3-deoxy-D-arabo-heptonic acid 7-phosphate which cyclizes to 5-dehydroquinic acid. This, in turn, proceeds to 5-dehydroshikimic acid and then to shikimic acid which, by way of its 5-phosphate, condenses with another mole of phosphoenolpyruvic acid to form prephenic acid. The latter aromatizes to yield phenylpyruvic acid (or its *p*-hydroxy derivative), then proceeds to phenylalanine (or tyrosine). Enzymatic deamination of phenylalanine, for example, provides cinnamic acid which has been shown to undergo *o*-hydroxylation followed by glucoside formation. The glucoside, in some manner, is finally converted to coumarin. The above sketch of the biosynthetic pathway to coumarins is admittedly brief; the reader is directed to the recent papers by Grisebach and Ollis (12) and Brown (13) for a more complete discussion together with key references. Brown points out that there are actually only a few of the simple coumarins about which much is known, largely through C^{14} tagging experiments. The growing activity in the field, however, is well illustrated by the work of Chambers, *et al.* (14), and Bunton, *et al.* (15), on the biosynthesis of novobiocin, and the work of Grisebach, *et al.* (16), on the biosynthesis of coumestrol.

Isolation Methods.—Ordinarily, the plant material is milled to a suitable state of subdivision and extracted in conventional types of apparatus. That the selection of the proper plant parts may be of great importance is emphasized by the comparative studies on isolation of visnadin by Smith, *et al.* (17), and Abu-Shady (18). In the former isolation, the total extract of the seeds of *Ammi visnaga* L. was worked up by a lengthy and tedious chromatographic procedure to obtain finally visnadin, together with its congeners, samidin, and dihydrosamidin. On the other hand, Abu-Shady utilized only the seed-free umbels and, by a relatively straightforward extraction and crystallization, obtained visnadin in good yield and in an excellent state of purity.

For the most part, isolation procedures depend upon successive extraction with solvents of increasing polarity. Thus, petroleum ether is frequently used as the initial extraction menstruum and has the advantage that most of the oxygenated coumarinic materials are not particularly soluble in it, although it must be pointed out that the oils extracted together with the coumarins tend to exert a solubilizing effect. A quick wash of the plant material with ice-cold petroleum ether (19) often removes much of this oil without undue sacrifice of coumarin material. As a result, coumarins quite frequently crystallize directly from the concentrated extract either during Soxhlet extraction or on standing and further concentration and cooling of the extract. Advantage, however, may be taken of the alkali hydroxide solubility and acid insolubility of most coumarins to separate them from other alkali-insoluble constituents in the extract. Plant acids are separated easily from coumarins by suitable use of sodium bicarbonate or sodium carbonate solutions which solubilize the acids only, the coumarins being soluble only in alkali hydroxides with or without the use of added alcohol. Petroleum ether may be omitted (and often is) and direct ether extraction may be employed. In either case, the above purification procedure may be used, although one must be cognizant of the possibility of facile hydrolysis of alkali-sensitive groups. This is particularly true of compounds such as samidin and its congeners (17) and also of pteryxin and suksdorfin (20). In these cases, a rapid expulsion of the 4′-acyloxy group takes place in the presence of alkali. Following extraction with petroleum ether and/or ether, the use of methanol or ethanol as the menstruum often results in the fortuitous crystallization of coumarinic glycosides. However, if no crystallization occurs, the glycosidic materials may be obtained by treatment of the extract in the usual way, such as the lead method, etc. In the event that the glycoside *per se* is not desired or is not obtainable, it is customary to cleave the coumarinic glycosides hydrolytically with acid or enzyme, then to recover the aglycones in the normal fashion already described. In any event, an effort should be made to identify the coumarins present in the extracts *before* chemical treatment so that comparisons can be made following such treatment to detect

possible hydrolytic cleavages. This is conveniently done with paper or thin-layer chromatographic techniques, taking advantage of the fluorescence of most coumarins under ultraviolet light. Isolation methods employing sublimation techniques are of limited usefulness due to the real possibility of structural modifications resulting in artifacts as a consequence of thermal lability. Nevertheless, the method is employed in the case of some of the simpler coumarins if care is taken to determine that the isolated products were originally present in the plant material.

Purification of crude coumarin fractions may be carried out by fractional crystallization techniques or by separations employing column chromatography, preparative thin-layer chromatographic techniques (21), or any of the other commonly used preparative chromatographic procedures. Examples of the power of the chromatographic methods in effecting separations are the isolations of Stanley, *et al.* (22), on lemon oil constituents and of Svendsen (23) in identifying the components of Norwegian umbellifers. A very helpful device in this respect has been the chromatostrip (24) for following the elution pattern from a column. The chromatostrip technique enables quick determinations of purity of fractions and is not unlike thin-layer chromatography, albeit less refined. Chromatography is a powerful means for demonstrating the homogeneity of unknown and known coumarins and frequently serves for identification purposes.

Chemical and Physical Properties.—Except

TABLE I.—GENERAL CHEMICAL REACTIONS OF COUMARIN NUCLEUS[a]

Reagent or Reaction Type	Result
Acids	No reaction. Solution in concentrated sulfuric acid often gives visible blue fluorescence
Ammonia	No reaction even under pressure and high temperatures
Hot dilute aqueous NaOH for short period of time	Formation of water-soluble yellow sodium coumarinate which regenerates original coumarin with acid
Hot dilute aqueous NaOH for prolonged heating period (or use of yellow HgO catalysis for shorter period of time)	Isomerization of initially formed sodium coumarinate (*cis*) to sodium coumarate (*trans*) which gives free coumaric acid instead of lactone on acidification
Alkali fusion or hot concentrated alkali	Usually provides benzenoid ring system as a phenol
Potassium carbonate and methyl iodide (or sulfate)	Methylates any phenolic hydroxyls but does not open coumarin ring
Sodium hydroxide and methyl iodide (or sulfate)	Forms *o*-methoxycinnamic acids
Reduction	
(*a*) H_2 with Pd/C	Smooth hydrogenation of 3:4 bond
(*b*) H_2 with Raney Ni (40–100°)	Hydrogenation of 3:4 bond
(*c*) H_2 with Raney Ni (200–250°)	Hydrogenation of entire ring system to give octahydrocoumarin and hexahydrochroman
(*d*) H_2 with Cu chromite	Reduction to 3-(*o*-hydroxyphenyl)-propyl alcohol
(*e*) Na and alcohol	Reduction to 3-(*o*-hydroxyphenyl)-propyl alcohol
(*f*) Na-Hg in dilute solutions	Reduces 3:4 bond to give sodium melilotate
(*g*) Na-Hg in concentrated solutions	Bimolecular reduction to give acid which provides dihydrodicoumarin on distillation
(*h*) Zinc and alkali (or AcOH)	Very similar to (*f*) and (*g*) above
(*i*) $LiAlH_4$	Yields *o*-hydroxycinnamyl alcohol and 3-(*o*-hydroxyphenyl)-propyl alcohol
Oxidation	
(*a*) Nitric acid or permanganates	Gives succinic acid if coumarin is first reduced at 3:4 position but none if not reduced (method for identifying coumarin system)
(*b*) Ozone	Order of attack is: double bonds in side chains, furan ring double bond, and finally 3:4 bond
(*c*) CrO_3	Resistant with respect to coumarin ring
(*d*) Permanganates	Usually results in a phenolic carboxylic acid
(*e*) Per acids	Resistant but forms epoxides of side chain double bonds
(*f*) Alkaline persulfates	Form 6-hydroxy coumarins
(*g*) Hydrogen peroxide (alkaline)	Especially useful with furanocoumarins, leaving furan-2:3-dicarboxylic acid
Mercuration	
(*a*) Mercuric chloride	Addition to 3:4 bond
(*b*) Mercuric acetate	Formation of 3,6,8-triacetoxymercuri-4-methoxydihydrocoumarin. This, on Br_2 treatment, gives 3,6,8-tribromocoumarin
Diazonium salts (with K_2CO_3)	Couple in 6 position
Grignard reagent	Adds first to carbonyl which may give an unsaturated ketone or, with excess reagent, can add a second mole to eventually give 2,2-dialkyl-1,2-benzopyrans or Δ^3-chromenes

for coumarin and dicumarol, all other naturally occurring coumarins bear an oxygen substituent at the 7 position (Table III) so that, in reality, one could say that umbelliferone (7-hydroxycoumarin) is the parent compound rather than coumarin. However, all coumarins are characterized by the benz-α-pyrone system which makes identification fairly straightforward in most cases. This lactonic character enables coumarins to be water-solubilized by alkali hydroxides with the usual appearance of yellow in the solution. The yellow coloration is particularly valuable for diagnostic purposes if alcohol is needed to bring the coumarin into solution sufficiently to enable alkali to react with it. Acidification (even with CO_2) of aqueous solutions results in prompt relactonization and recovery of the original coumarin barring hydrolytic cleavages. The speed of relactonization is such that it is virtually impossible in most cases to obtain the intermediate *o*-hydroxycinnamic acid unless a hydrogen bonding group such as nitro or carbonyl is present in the 8 position to slow down the process by bonding with the phenolic hydroxyl group.[a] Many of the other chemical properties of coumarins are given in Table I which summarizes most of those reactions common to this nucleus.

An interesting property of coumarins is their ability to fluoresce under ultraviolet light. Fluorescence of coumarins is not necessarily a unique feature of this group compared to other plant constituents but is, nevertheless, a useful diagnostic feature when used properly. The studies of Goodwin and Kavanagh (25, 26) on fluorescence as a function of pH are interesting in this respect as are those of Feigl, *et al.* (27), on

TABLE I.—(continued)

Reagent or Reaction Type	Result
Hydroxylamine	Adds 3 moles of NH_2OH in absence of alkali or 2 moles in presence of alkali to give a hydroxamic acid. Added $FeCl_3$ in latter case gives deep violet color (test for coumarins)
Diazomethane	Usually methylates only phenolic hydroxyls but can also form a pyrazoline derivative across 3:4 bond
Sodium bisulfite	Bisulfite addition compound at 4 position
Halogenation	
(*a*) Cl_2 or Br_2	Adds to 3:4 bond and then alkali will remove HX to give a 3-halogenocoumarin. Alkali converts the latter to coumarilic acids
(*b*) *N*-Bromosuccinimide	Forms 3-bromocoumarin directly
Nitration	Principally at 6 position; small amount at 8 position
Light	Causes photodimerization in some cases
Phosphorus pentasulfide	Forms thiocoumarins that are readily converted to the oxime and phenylhydrazone of the coumarin; otherwise unobtainable from coumarin directly
Diels-Alder conditions	Adds butadienes across 3:4 bond but addition is better to *o*-methoxycinnamic acids
Michael reaction conditions	Adds cyanoacetamide, malonic ester, and ethylphenylacetate
Special reactions of 7-hydroxycoumarin (umbelliferone)	
(*a*) Duff reaction	Forms 8-formyl-7-hydroxycoumarin
(*b*) Claisen migration on 7-allyloxycoumarin	Forms 8-allyl-7-hydroxycoumarin
(*c*) Fries rearrangement of esters of 7-hydroxycoumarin	Forms 8-acyl-7-hydroxycoumarin which on alkali treatment yields 2-acylresorcinols (Nidhon synthesis)
Special reactions of 4-hydroxycoumarin (benzotetronic acid)	
(*a*) Bicarbonates	Confer water solubility
(*b*) Aniline	Forms 4-anilino derivative
(*c*) PX_5	Forms 4-halogeno derivative
(*d*) Diazonium salts	Couple at 3 position to give 3-phenylhydrazone
(*e*) Aldehydes	Addition due to active methylene at 3 position
(*f*) Hydrolysis with aqueous alkali	Mild conditions give *o*-hydroxyacetophenone; more rigorous conditions give salicylic acid
(*g*) $\alpha\beta$-Unsaturated ketones	Adds in Michael manner
(*h*) Diazomethane	Mainly methylation of 4-hydroxyl but small amount of 2-methoxychromone also forms due to tautomeric 2-hydroxychromone
(*i*) Ferric chloride	No color in spite of enolic character due to unfavorable steric disposition for complex formation
(*j*) Hydrogenation	Difficult but proceeds to 4-hydroxydihydrocoumarin which dehydrates readily to reform the coumarin

[a] For a comprehensive survey of coumarin chemistry see Wawzonek, S., "Heterocyclic Compounds," Vol. 2, edited by Elderfield, R. C., John Wiley and Sons, Inc., New York, N. Y., 1951, p. 173.

the use of fluorescence *via* photocatalysis as a sensitive and specific test for coumarin. Ichimura (28) has published the spectra of numerous coumarins, and Wheelock (29) has studied the relationship of structure to fluorescent properties in an extensive series of coumarins. Crosby and Berthold (30) describe the paper chromatography of several coumarins and the usefulness of fluorescence spectra for characterizing the eluted coumarins. Most coumarins fluoresce blue under ultraviolet light and many of the 7-oxygenated ones even fluoresce in visible light, especially if dissolved in concentrated sulfuric acid. In alkaline solution the fluorescence usually turns green and may even disappear, particularly when the hydroxyl at the 7 position is free. A greenish fluorescence is noted with many of the furanocoumarins in a neutral state. Fluorescence is not particularly enlightening in determining structural features but is very helpful in locating and recovering coumarin spots on a chromatogram without use of a chemical spray.

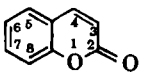
Because coumarins have well defined absorption bands in the ultraviolet region, they have been studied extensively in the hope that these spectra might have structural significance. The ultraviolet absorption pattern of the coumarin nucleus can be attributed largely to the benz-α-pyrone structure and Nakabayashi, *et al.* (31), assume that the two absorption bands of coumarin at 270 mμ and 312 mμ are those of benzene at *ca.* 200 mμ and 240–260 mμ that had shifted to these regions. The α-pyrone moiety absorbs at about 300 mμ (log ϵ, *ca.* 4), and variations from this are attributed to the pronounced effect of substituents and their location. Ganguly and Bagchi (32) considered the spectra of coumarins and chromones with a view toward finding differences that would eliminate confusion between these two superficially similar groups. They noted that chromones have strong absorption at 240–250 mμ with a log ϵ over 3.8, whereas the coumarins usually have a deep minimum at this wavelength. With respect to nonhydroxylated coumarins, they found that methyl substitution had little effect on the positions of the parent absorption bands. On the other hand, Sen and Bagchi (33) found that introduction of a hydroxyl group into the nucleus caused a general bathochromic shift of the principal absorption bands. In assessing the differences between hydroxychromones and hydroxycoumarins they found that the former show a considerable amount of absorption around 250 mμ with the log ϵ invariably over 4.1, whereas the log ϵ in the case of the hydroxycoumarins was never over 3.75. Furthermore, the minima of the hydroxycoumarins were between 255–262 mμ, but those of the hydroxychromones were over 280 mμ. There have been many studies (22, 34–42) on the ultraviolet spectral characteristics of the coumarins and, because of the variations shown by the naturally occurring variety, it is desirable to consult the original papers cited for specific details. One point that is worth mentioning is the bathochromic shift that usually ensues together with an increase in the extinction coefficient when the spectrum of a given coumarin is taken in an alkaline medium. For example, the maximum of umbelliferone at 325 mμ (log ϵ, 4.15) shifts to 372 mμ (log ϵ, 4.23) in alkaline solution.

Infrared analysis has found its place in the structural characterization of coumarins also. The particular value of such analysis, aside from detecting groups that are not fundamentally related to the coumarin nucleus, lies in its ability to assign a lactone function to the coumarin. Characteristic bands for the α-pyrone moiety are found, as a rule, in the region of 1715–1745 cm.$^{-1}$ together with the conjugated aromatic double bond at about 1625–1640 cm.$^{-1}$ Aromatic absorption bands are found in the usual regions.

High resolution nuclear magnetic resonance (NMR) spectra also have been of use in the structural definition of coumarins. In some cases, the structural argument has been based almost solely on the NMR spectrum (43, 44). In other cases, this analytical procedure has been used to assign a structure where it would have been difficult or impossible to do it chemically (45, 46). Dharmatti, *et al.* (47), have examined the high-resolution NMR spectra of coumarin and a number of its derivatives and have noted that the calculated and observed chemical shifts and coupling constants for the four protons in the benzene ring of coumarin agree satisfactorily. The chemical shifts for the protons in the 3 and 4 positions of coumarin are almost the same as those observed for the ethylenic protons in *o*-coumaric acid; thus, they do not substantiate the earlier view of a naphthalene-type resonance in coumarins (48). The coupling constant ($J_{3,4}=9.8$) confirms that the protons at the 3 and 4 positions are *cis* to each other as expected. They point out that the NMR spectra provide a convenient method for distinguishing between 3 and 4 substituted coumarins on the basis of the observed chemical shifts.

The use of mass spectrometry in coumarin

TABLE II.—NATURALLY OCCURRING COUMARINS



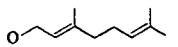
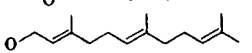
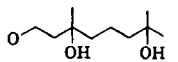
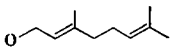
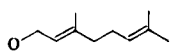
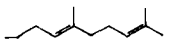
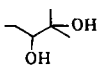
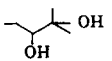
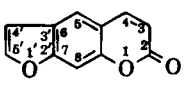
No.	Name (Synonyms)	M.p., °C.	Position of Substituent 4	5	6	7	8	$[\alpha]_D$ (solv., °C.)	Refs.
					A. Simple Coumarins				
1.	Coumarin	67–68	H	H	H	H	H	...	(5)
2.	Umbelliferone (skimmetin, dichrin A)	223–224	H	H	H	OH	H	...	(163–167)
3.	Skimmin	219–221	H	H	H	O-Glucoside	H	−79.8 (pyr., 18)	(168, 169)
4.	Neohydrangin	204	H	H	H	O-Diglucoside	H	−130.9 (H_2O, 21)	(170)
5.	Herniarin (ayapanin)	114	H	H	H	OCH_3	H	...	(165, 171, 172)
6.	Umbelliprenin	61–63	H	H	H		H	...	(173)
7.	Auraptene	68	H	H	H		H	...	(174, 175)
8.	Marmin	125	H	H	H		H	...	(176, 177)
9.	Esculetin (cichorigenin, crategin, aesculetin)	268–272 dec.	H	H	OH	OH	H	...	(164, 178–182)
10.	Esculin (aesculin)	205	H	H	O-Glucoside	OH	H	−146 (CH_3OH, 15)	(178, 179, 183, 184)
11.	Cichoriin	213–215	H	H	OH	O-Glucoside	H	−104.5 (dioxan)	(185, 186)
12.	Scopoletin	205–205	H	H	OCH_3	OH	H	...	(187–190)
13.	Scopolin	217–219	H	H	OCH_3	O-Glucoside	H	...	(187, 191, 192)
14.	Angelical	256–257	H	H	CHO	OCH_3	H	...	(193)
15.	Glabralactone	129–130	H	OCH_3	H	OCH_3		...	(194, 195)
16.	Fabiatrin	236–238	H	H	OCH_3	O-Primveroside	H	−140 (H_2O, 20)	(196)
17.	Ayapin	231–232	H	H	O—CH_2—O		H	...	(197, 198)
18.	Esculetin dimethyl ether (scoparone, scoparin)	144–146	H	H	OCH_3	OCH_3	H	...	(180, 199)
19.	Daphnetin	255–256 dec.	H	H	H	OH	OH	...	(200–202)
20.	Daphnin	223–224 dec.	H	H	H	O-Glucoside	OH	−123.9 (CH_3OH, 22)	(202–206)
21.	Daphnetin glucoside	197–198	H	H	H	OH	O-Glucoside	+4.57 (CH_3OH, 23)	(207)
22.	Citropten (limettin)	146–147.5	H	OCH_3	H	OCH_3	H	...	(208–211)
23.	5-Geranoxy-7-methoxy coumarin	86–87	H		H	OCH_3	H	...	(212)
24.	Collinin	67–68	H	H	H		OCH_3	...	(213)

TABLE II.—(*continued*)

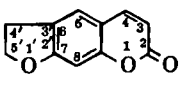
No.	Name (Synonyms)	M.p., °C.	Position of Substituent 4	5	6	7	8	[α]D (solv., °C.)	Refs.
25.	Fraxinol	171–173	H	OCH_3	OH	OCH_3	H	...	(214)
26.	Fraxetin	227–228	H	H	OCH_3	OH	OH	...	(215–218)
27.	Fraxin (paviin)	205	H	H	OCH_3	OH	O-Glucoside	...	(215, 219, 220)
28.	Fraxidin	196–197	H	H	OCH_3	OCH_3	OH	...	(217, 220, 221)
29.	Isofraxidin (calycanthegenol)	148–149	H	H	OCH_3	OH	OCH_3	...	(217, 221)
30.	Calycanthoside (isofraxidin glucoside)	219–220	H	H	OCH_3	O-Glucoside	OCH_3	−42° (CH_3OH)	(222)
31.	6,7,8-Trimethoxy coumarin	104	H	H	OCH_3	OCH_3	OCH_3	...	(216, 223)
32.	Osthenol	124–125	H	H	H	OH		...	(224)
33.	Vellein	187.5–189	H	H	H	O-Glucoside		...	(225)
34.	Osthol	83–85	H	H	H	OCH_3		...	(226–228)
35.	Merangin (auraptene)[a]	98	H	H	H	OCH_3		−33.4 (C_2H_5OH, 20)	(5, 229, 332, 333)
36.	7-Demethylsuberosin	133.5–134	H	H		OH	H	...	(223)
37.	Suberosin	87–88	H	H		OCH_3	H	...	(230)
38.	Ostruthin	117–119	H	H		OH	H	...	(231–234)
39.	Geijerin	121	H	H		OCH_3	H	...	(235, 236)
40.	Toddaculine	95	H	OCH_3		OCH_3	H	...	(237)
41.	Angelicone	130	H	OCH_3		OCH_3	H	...	(238)
42.	Toddalolactone	131–132.5	H	OCH_3		OCH_3	H	+55.9 ($CHCl_3$, 30)	(239, 240)
43.	Aculeatin hydrate[b]	131–132.5	H	OCH_3		OCH_3	H	+50.9 ($CHCl_3$, 26)	(241)
44.	Aculeatin	113	H	OCH_3		OCH_3	H	−16.8 (EtOAc, 24)	(241)
45.	Brayleyanin	95	H	H	OCH_3			...	(242)

46.	Dalbergin	209–210	C_6H_5	H	OH	OCH_3	H	...	(243–245)
47.	Mammein	128.5–129	*n*-C_3H_7	OH		OH		...	(246–248)
48.	4-Phenyl-5,7-dihydroxy-6-isovaleryl-8-isopentenyl coumarin	98–109	C_6H_5	OH		OH		...	(249–250)

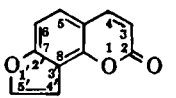
B. Furanocoumarins



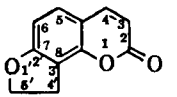
Psoralen Type



Dihydropsoralen Type



Angelicin Type



Dihydroangelicin Type

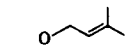
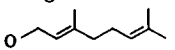
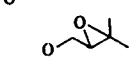
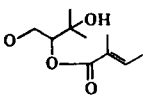
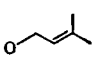
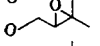
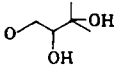
			Psoralen Type					
			Position of Substituent					
			5	4′	5′	8		
49.	Psoralen	161–163	H	H	H	H	...	(78, 251)
50.	Bergaptol	278–282	OH	H	H	H	...	(212, 252, 253)
51.	Bergapten (heraclin, majudin)	188–191 (sub.)	OCH_3	H	H	H	...	(254–257)
52.	Isoimperatorin	109		H	H	H	...	(258, 259)
53.	Bergamottin	59–61		H	H	H	...	(234, 260)
54.	Oxypeucedanin	141–143		H	H	H	Slightly levorotatory	(232, 261, 262)
55.	Ostruthol	136–137		H	H	H	−18.3 (pyrid., 15)	(263)
56.	Xanthotoxol	249–251	H	H	H	OH	...	(264–266)
57.	Xanthotoxin	145–146	H	H	H	OCH_3	...	(267–269)
58.	Prangenin	97	H	H	H	OC_4H_9(*n*)	...	(270, 271)
59.	Imperatorin(marmelosin, ammidin)	102–105	H	H	H		...	(264, 266, 272)
60.	5-Methoxy-8-hydroxy-psoralen	210–212	OCH_3	H	H	OH	...	(273)
61.	Isopimpinellin	148–151 dec.	OCH_3	H	H	OCH_3	...	(274, 275)

TABLE II.–(*continued*)

No.	Name (Synonyms)	M.p., °C.	Position of Substituent 5	4′	5′		[α]D (solv., °C.)	Refs.
62.	Phellopterin	102	OCH_3	H	H		...	(276, 277)
63.	Byakangelicol	106	OCH_3	H	H		+34.77 (pyrid., 25)	(276–278)
64.	Ferulin[d]	87	OCH_3	H	H		+27.31 (23) (pyrid., 23)	(279)
65.	Byakangelicin	125–126	OCH_3	H	H		+24.62 (pyrid., 25)	(276, 280)
66.	Psoralidin[e]	315 dec.	OH	H	H		...	(281, 282)
67.	Peucedanin (oreoselone methyl ether)	109	H	OCH_3			...	(283, 285)

DIHYDROPSORALEN TYPE

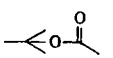
68.	Nodakenetin	185–192	H	H		H	−25.4 ($CHCl_3$, 24)	(286, 287, 368)
69.	Marmesin[f]	189.5	H	H		H	+26.8 ($CHCl_3$, 34)	(288, 289, 368)
70.	Nodakenin	215–219	H	H		H	+56.6 (H_2O, 30)	(286, 287)
71.	Marmesinin	259–260 dec.	H	H		H	−60 (50% C_2H_5OH, 25)	(290, 291)

ANGELICIN TYPE

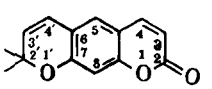
			Position of Substituent 5	6	5′	4′		
72.	Angelicin (isopsoralene)	138–140	H	H	H	H	...	(292, 293)
73.	Isobergapten	218–222	OCH_3	H	H	H	...	(253, 294)
74.	Sphondin	192–193	H	OCH_3	H	H	...	(295, 296)
75.	Sphondylin	161–163	A monomethoxyfuranocoumarin of uncertain structure, isomeric with No. 73 and 74				...	(295)
76.	Pimpinellin	117–119	OCH_3	OCH_3	H	H	...	(275 297)

DIHYDROANGELICIN TYPE

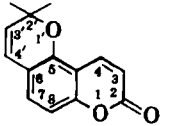
77.	Columbianetin	164.5–165	H	H		H	+20 (diox., 27)	(46)
78.	Columbianin	275–276(with $2H_2O$)	H	H		H	+118 (H_2O, 23)	(46)
79.	Columbianadin	121–122	H	H		H	+26.5 (diox., 27)	(46)
80.	Discophoridin	134–135	H	H		H	+20.4 (C_2H_5OH, 20)	(43)
81.	Athamantin	58–60	H	H			+96 (CH_3OH, 22)	(39, 298–300)

82.	Edultin	138-140	H	H			+41.13 (pyrid., 10.5)	(301)

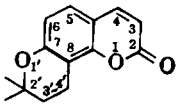
C. Pyranocoumarins (Chromeno-α-pyrones)



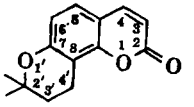
Xanthyletin Type



Alloxanthyletin Type



Seselin Type

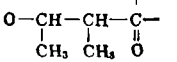


Dihydroseselin Type

XANTHYLETIN TYPE

			Position of Substituents 4	5	8		
83.	Xanthyletin	128–131	H	H	H	...	(302–304)
84.	Xanthoxyletin	132	H	OCH_3	H	...	(305, 306)
85.	Luvangetin	108–109	H	H	OCH_3	...	(307–309)

ALLOXANTHYLETIN TYPE

			Position of Substituents 4	7	8		
86.	Alloxanthoxyletin	115.5	H	OCH_3	H	...	(310)
87.	Calophyllolide	158	$-C_6H_5$	OCH_3		...	(311, 312)
88.	Inophyllolide	188	$-C_6H_5$			...	(312)

SESELIN TYPE

			Position of Substituents 5	6	3′	4′		
89.	Seselin	119–120	H	H	H	H	...	(313–315)
90.	Braylin	150	H	OCH_3	H	H	...	(242)

DIHYDROSESELIN TYPE

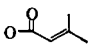
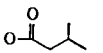
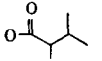
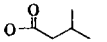
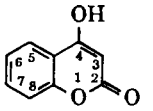
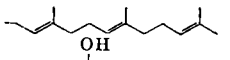
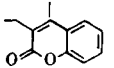
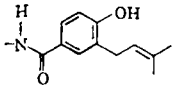
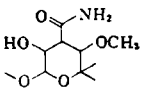
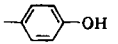
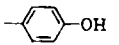
91.	Samidin	138–139	H	H		$OOC-CH_3$	+100 (dioxan)	(17, 316)
92.	Dihydrosamidin	111–113	H	H		$OOC-CH_3$	+63 (dioxan)	(17)

TABLE II.—(*continued*)

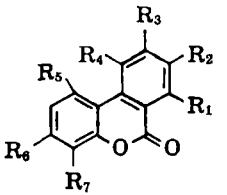
No.	Name (Synonyms)	M.p., °C.	Position of Substituent 5	6	3′	4′	[α] (solv., ° C.)	Refs.
93.	Visnadin (provismine)	85–88	H	H		OOC–CH₃	+38 (dioxan)	(17, 18, 316)
94.	Suksdorfin	140.5–141	H	H	OOC–CH₃		+4 (C_2H_5OH, 24)	(20, 317)
95.	Pteryxin	81.5–82.5	H	H	OOC–CH₃		+10 (C_2H_5OH, 22)	(20, 318)

D. 4-Hydroxycoumarins



No.	Name (Synonyms)	M.p., °C.	Position of Substituents 3	5	7	8	[α] (solv., ° C.)	Refs.
96.	Ammoresinol	107–108		H	OH	H	...	(319–322)
97.	Dicumaro	288–289		H	H	H	...	(49, 323)
98.	Novobiocin (albamycin, cathomycin, cardelmycin, spheromycin, etc.)	...		H		CH_3	...	(324–329)
99.	Isoshekkangenin[g]	228		OHC_3	OH	H	...	(330, 331)
100.	Isoshekkanin[g]	253		OCH_3	O–Glucoside	H	...	(330)

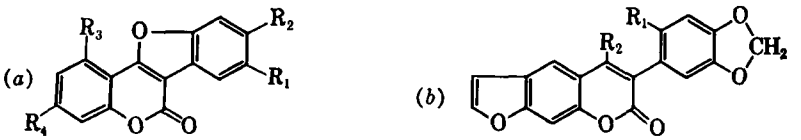
E. 3,4-Benzocoumarins



No.	Name (Synonyms)	M.p., °C.	Position of Substituents R_1	R_2	R_3	R_4	R_5	R_6	R_7	[α] (solv., ° C.)	Refs.
101.	2′:3″-Dihydroxydibenz-α-pyrone	360 dec.	H	OH	H	H	H	OH	H	...	(9)

102.	Alternariol	350 dec.	OH	H	OH	H	CH_3	OH	H	...	(334)
103.	Alternariol methyl ether	267	OH	H	OH (or OCH_3)	H	CH_3	OCH_3 (or OH)	H	...	(334)
104.	3:5:3′:5′-Dihydroxydiphenic acid dilactone	360	H	OH	H	O——OC		OH	H	...	(9)
105.	3:5:3′:5′-Dihydroxy-4:4′-dimethoxydiphenic acid dilactone	337–338	H	OH	OCH_3	O——OC		OH	OCH_3	...	(9, 335)
106.	Ellagic acid	360	H	OH	OH	O——OC		OH	OH	...	(9, 336, 337)

F. 3 Phenylcoumarins (and derivatives)



(*a*)

		Position of Substituents					
		R_1	R_2	R_3	R_4		
107.	Coumestrol	H	OH	H	OH	...	(68–70)
108.	Wedelolactone	OH	OH	OH	OCH_3	...	(338, 339)

(*b*)

		Position of Substituents				
		R_1	R_2			
109.	Pachyrrhizin	OCH_3	H		...	(340)
110.	Erosnin	O			...	(341, 342)

G. Miscellaneous Coumarins

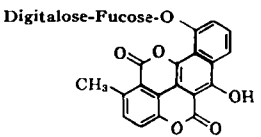
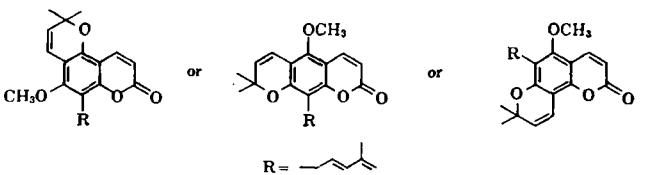
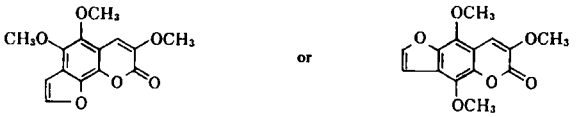
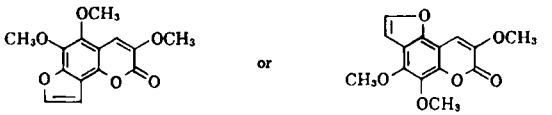
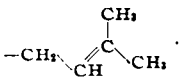
			Structure(s)		
111.	Chartreusin (antibiotic X-465A)	184–186		+132 (pyr., 25) −33 (gl. acetic acid, 25)	(343–347)
112.	Avicennin	141–142		...	(45, 348–350)

TABLE II.—(*continued*)

No.	Name (Synonyms)	M.p., °C.	Structures	[α]D (solv., °C.)	Refs.
113.	Halfordin	136–137		...	(351)
114.	Isohalfordin	151–152		...	(351)
115.	Aflatoxin B (I) Aflatoxin G (II)	268–269 244–246	 I II	... ...	(8, 44)

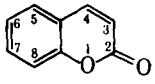
[a] See also entry No. 7. Priority of the name rests with No. 7. [b] Aculeatin hydrate is identical to No. 42 or is an isomer. [c]  is to be interpreted as  . [d] Formula claimed is that of No. 63; matter has not been cleared up yet. Very doubtful that this formula is correct (see *Reference 282*). [f] No. 69 is the optical isomer of No. 68. [g] The validity of this structure is doubtful.

structural studies has not been exploited to any degree yet. However, the technique has been used to determine the exact molecular weights of compounds in scarce supply (44). Molecular weights may be obtained also by X-ray determination of the unit-cell dimensions for a given compound which, together with the density of the crystals, enables a precise calculation of this value that can be very essential to structural elucidation. The latter technique has been helpful in the determination of the structure of novobiocin, a coumarin-type antibiotic.

Classification.—There are several structural categories into which the coumarins could be divided based on the desires and needs of the classifier. The classification employed in Table II suffices for the purposes of this review. It structurally defines the known coumarins and gives some of the pertinent physical constants and references to the original literature. The various complex coumarins in the first category, *Simple Coumarins*, may indicate something of an overstatement insofar as the connotation of simplicity is concerned, when one considers the relative complexity of some of the entries. Nevertheless, for ease of classification, this group was handled best in this manner. The other classes, *i.e.*, *furanocoumarins*, *pyranocoumarins*, 4-*hydroxycoumarins*, 3,4-*benzocoumarins*, and 3-*phenylcoumarins* have relatively straightforward structural relationships. The various structural features to be found in natural coumarins are recorded in Table III and illustrate the variety of substituents and their positions to be found in this group.

Physiological Activity.—Although the naturally occurring coumarins are a large and important group of plant products, it is doubtful

TABLE III.—GENERAL STRUCTURAL FEATURES OF NATURAL COUMARINS



Position	Substituent	Comment
3	—AR—	Aromatic ring fused across 3:4 bond
	—AR	Phenyl ring, usually phenolic. May or may not be bridged at an *o*-position to the 4 position by an ether linkage)
	—R	Farnesyl
	—OR	Methoxyl (only in halfordin and isohalfordin)
	—CO—$(CH_2)_2$—	Bridged to 4 position
	—COO$(CH_2)_2$—	Bridged to 4 position
	—CH_2-coum.	Bis compound (see dicumarol)
	—NHCO—R	See novobiocin
4	—OH	High degree of enolic character
	—R	Only *n*-propyl (see mammein)
	—AR	Phenyl
	—AR—	Fused across 3:4 position
5	—OH	Typically phenolic
	—OR	(*a*) Methoxyl, isopentenyloxy (and its epoxide and hydrated epoxide), and geranoxy (*b*) Part of pyrano ring (and possibly furano) attached to 6 position
	—R	Methyl (only in alternariol and its methyl ether)
	—COO—	Lactone bridge to benzene ring fused at 3:4 position (see ellagic acid and congeners)
6	—OH	Typically phenolic
	—OGl	Glucoside
	—OR	Methoxyl, and methylenedioxy (to 7 position)
	—R	(*a*) Isopentenyl (and its epoxide and hydrated epoxide) and geranyl (*b*) Point of attachment of pyrano or furano ring system originating at 5 or 7 position
	—CO—R	Isopentenoyl and isopentanoyl (isovaleroyl)
7	—H	Found only in coumarin and dicumarol
	—OH	Typically phenolic. Present in all natural coumarins as free OH or one of the combined forms below
	—OGl	Glucoside (or, in one case, a primveroside)
	—OR	(*a*) Methoxyl, methylenedioxy (to 6 position), isopentenyloxy, geranoxy (and its dihydroxy derivative), and farnesyloxy (*b*) Part of ring system attached as furano or pyrano type to 6 or 8 position
8	—OH	Typically phenolic
	—OGl	Glucoside
	—OR	Methoxyl, *n*-butoxyl, isopentenyloxy (and its epoxide and hydrated epoxide)
	—R	Isopentenyl (and its epoxide and hydrated epoxide)
	—CO—R	Isopentanoyl (isovaleroyl)

that their full range of physiological actions has been appreciated by most investigators. There has been, however, a continuing effort and a buildup of literature in this area with an occasional sporadic flurry of publication concerned with certain coumarins that have caught the wider interest of scientists. Bose (7), as previously mentioned, has considered the biochemical properties of the natural coumarins in an effort to show that these products are not mere metabolic products of the living cell, but that they possess varied and often remarkable physiological actions. Thereby, he stimulated an interest in coumarins for others. In surveying the literature, the present author has succumbed to the temptation to depart from the announced intention of covering only naturally occurring coumarins and has, to a limited extent, covered some activities of synthetic coumarins that seemed of interest. Table IV summarizes the various physiological activities that are known to exist in the coumarin compounds with particular emphasis on activity associated with the natural products. A selected group of physiological activities will be discussed in more or less detail; the remainder, which represents only one or two investigations at best, will be taken care of by simply referring to the work.

ANTICOAGULANT ACTIVITY

Recognition of the coumarin moiety as an anticoagulant species dates back to the report of Link, *et al.* (49), concerning studies on the toxic agent of spoiled sweet clover hay. The often fatal toxic material causing serious hemorrhagic conditions in cattle was found to be 3,3′-methylenebis-(4-hydroxycoumarin) (*Dicumarol*, Table V). Following this discovery, the problem of structure-activity relationships was examined by numerous investigators, prominent among which are Link (50), Mentzer, *et al.* (51), Chmielewska and Cieslak (52), and Arora and Mathur (53). Link came to the conclusion that the minimum requirements for hypoprothrombinemic activity were an intact 4-hydroxycoumarin with a substituent at position 3, a keto group on the 3-substituent in a 1:5 position to the 4-hydroxyl and, for maximal activity, a bis arrangement was necessary. Mentzer and his co-workers also felt that this arrangement was needed, although subsequent work has shown that this is a nonessential feature. These early studies, of course, led to synthetic efforts in other laboratories, and today there is a substantial literature connected with compounds of this nature. The most important of these synthetic discoveries are listed in Table V. Most of the synthetic work has been directed toward correcting the shortcomings of the naturally occurring prototype, dicumarol, although it still enjoys extensive usage. The principal faults were a slow onset of action and too prolonged an action which was difficult to terminate—although the action could be stopped with vitamin K (or menadione), provided the dicumarol level was not too high (54). It may be observed, however, that the coumarins as a group are singularly free of toxic side reactions and may be given for years without development of untoward effects except hemorrhage from overdosage. It is worth noting that the activity of 3-(α-acetonylbenzyl)-4-hydroxycoumarin (warfarin), for example, was so great that it was employed as an effective hemorrhagic rodenticide for years before its acceptance into human

TABLE IV.—SOME PHARMACOLOGICAL AND PHYSIOLOGICAL PROPERTIES OF COUMARINS

Activity	Ref.
1. Inhibition of germination and root growth ("blastocholine effect"), *i.e.*, plant growth regulation	(7)[a]
2. Cytogenetic and cytological properties	(7, 352–354)
3. Curare-like action	(7)
4. Spasmolytic action	(7, 20, 317, 318, 355)
5. Narcotic effect	(7)
6. Sedative and hypnotic effects	(7) text[b]
7. Central nervous system paralysis	(7)
8. Central nervous system stimulation	(7, 356)
9. Respiratory stimulation	(357)
10. Uricosuric action	(7)
11. Vitamin-P-like action on capillary fragility	(7)
12. Anticoagulant action	(7) text
13. Hemostatic effect	(7)
14. Bacteriostatic, tuberculostatic, and antifungal action	(7) text
15. Anthelmintic action	(7) text
16. Piscicidal action	(7, 358–360)
17. Dermal photosensitization	(7) text
18. Antileucodermic action	(7) text
19. Bacterial photosensitization	text
20. Antiveratrinic action	(361)
21. Anti-retinal pigment degeneration effect	(367)
22. Analgesic action	text
23. Hypercholesteremic effect	(362)
24. Hypocholesteremic effect	(363)
25. Antidigitoxin activity	(364)
26. Hypothermal action	text
27. Estrogenic action	text
28. Molluscacidal action	text
29. Vasodilator action	text
30. Human chorionic gonadotrophin inhibitory action	(365)
31. Choleretic action	(366)

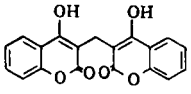
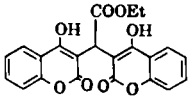
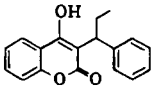
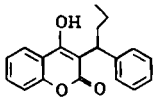
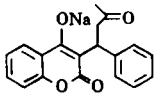
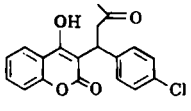
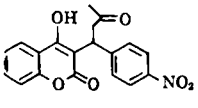
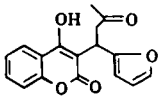
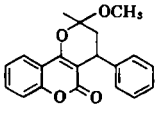
[a] *Reference* 7 gives a relatively recent survey of biochemical properties associated with coumarins and, in this table, reference to it suggests that the reader consult the original article for leading references. [b] "Text" indicates that an adequate discussion together with pertinent references is in this review.

therapy (*i.e.*, as coumadin). An interesting note in connection with racemic warfarin is that recently it has been resolved, its absolute configuration determined, and that levorotatory (S)-warfarin has been shown to be seven times as active as (R)-warfarin (55).

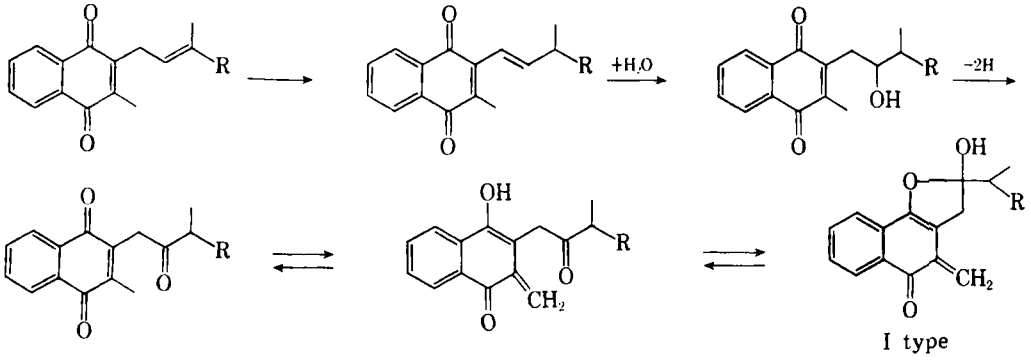
The activity associated with the coumarin-type molecule is currently (56) considered to be a vitamin K antagonism in which the anticoagulant competes with vitamin K in its blood clotting role. Thus, the clotting time is increased *in vivo* (but not *in vitro*) by a decrease in the prothrombin concentration of the blood presumably by interfering with its production in the liver. Prothrombin production is believed to arise from a combination of vitamin K (a compound having a 2-methyl-1:4-naphthoquinone nucleus) with an apoenzyme (AE) to form the active enzyme (AEK). The K vitamin acts as the prosthetic group of the enzyme. The coumarins (B) can compete successfully with the K vitamin for the apoenzyme by virtue of structural similarity and thus exert antivitamin K activity. The equation, $AEK + B \rightleftarrows AEB + K$, expresses this relationship as a reversible system and, thus, vitamin K can reverse the coumarin activity if present in sufficient concentration. Reduction of prothrombin production, of course, ensues if the form AEB predominates.

In keeping with this concept, Chmielewska and Cieslak (52) have hypothesized that the

TABLE V.—SOME USEFUL ANTICOAGULANT COUMARINS

Structure	Generic Name	Proprietary Name(s)
	Bishydroxycoumarin	Dicumarol
	Ethyl biscoumacetate	Tromexan ethyl acetate, pelentan
	...	Marcumar, marcoumar
	...	Liquamar
	Warfarin (sodium)	Coumadin (sodium)
	Coumachlor	Tomorin
	Acenocoumarol	Sintrom
	...	Fumarin
	Cyclocoumarol	Cumopyran

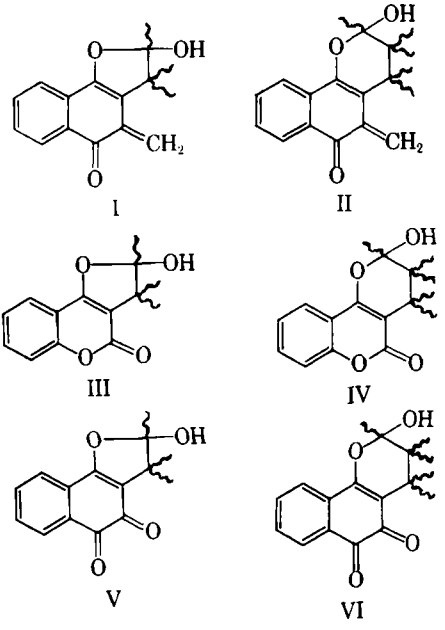
structure necessary for vitamin K activity is expressed by formulas I and II with formula I type arising from biological oxidation of the phytyl or difarnesyl chain of vitamins K as

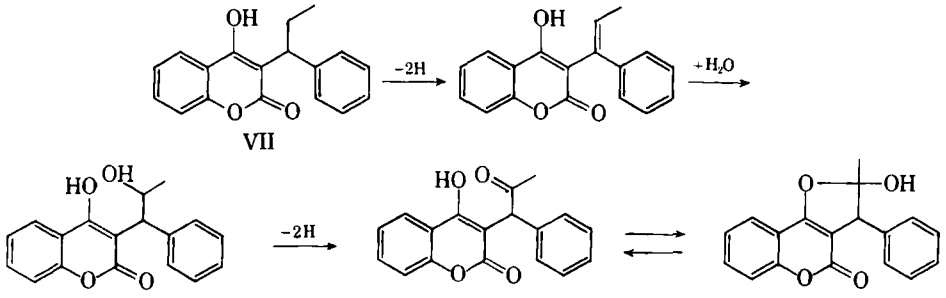


The problem associated with adapting dicumarol, in the face of the accepted bis-formula, was ingeniously solved by showing that dicumarol actually existed in a tautomeric keto-enol form

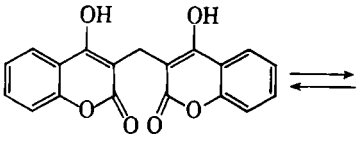
They further postulated that the *o*-quinoid group, O=C—C=CH_2, acts as the active center, whereas the hemiketal group is used to attach the active molecule to the protein part of the enzyme. In keeping with this hypothesis, they suggest that coumarin type antivitamin K compounds (III, IV) as well as V and VI (3-substituted derivatives of 2-hydroxy-1:4-naphthoquinone) which are known hemorrhagic agents lack the active center but contain the hemiketal linkage and, being similar in shape, successfully compete with vitamins K for the apoenzyme. Two active coumarins, however, failed to have the structural requisites to fit into their hypothesis—namely, 3-(1′-phenyl-*n*-propyl)-4-hydroxycoumarin (VII) and dicumarol itself. The known activity of VII was suggested by the authors as accounted for by a probable biological oxidation at the 2′-position, a postulation that was supported by their preparation of model compounds incapable of oxidation at the 2′-position, all of which were inactive. The suggested sequence of events in the proposed biological oxidation is

(VIII) which would, of course, leave the way open for formation of a hemiketal structure

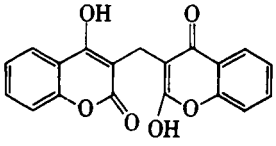




related to formula IV. In conformity to this,



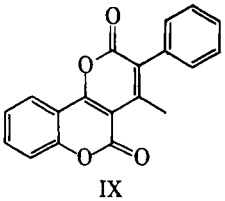
Dicumarol



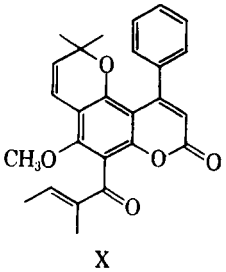
VIII

one can cite Link's cumopyran (see Table V) as an active agent.

Although the findings of Chmielewska seem rather conclusive, Arora and Mathur (53) have studied a series of coumarins recently with a view toward further amplification of the structure-activity relationships. In general, their findings support the Chmielewska-Cieslak hypothesis insofar as the 4-hydroxycoumarins are concerned. They found, however, that introduction of a methoxyl group into the 4-hydroxycoumarin molecules (including dicumarol) potentiated activity but that additional loading of the molecule with methoxyl groups could inhibit or augment activity, depending on the location of the groups. In particular, they felt that a methoxyl at position 8 had a special potentiating effect. A single compound (IX) in their series, at least formally related to formula IV, showed considerable activity, although they felt their observation to be on such a limited basis that it had little significance. Calophyllolide (X), however, with an activity superior



IX



X

to that of Dicumarol is unexplainable on the basis of the Chmielewska-Cieslak hypothesis. This naturally occurring coumarin apparently has excellent anticoagulant activity (53) but is not a 4-hydroxycoumarin, nor does it have a substituent in the 3-position. Arora and Mathur suggest that the inhibitory effect of the 4-phenyl group (noted in their studies on 4-phenylcoumarins) is probably counteracted by several potentiating factors which presumably are the 7-methoxyl, the 8-acyl group, and the additional dimethylpyrano group bridging positions 5 and 6. In conclusion, the latter authors state that anticoagulant activity is "governed not by individual structural features but by a combination of several, none of which can be defined precisely at the present time." Among the general structural features that could be important, however, they cited molecular shape, a six-membered heterocyclic ring (other than pyrone) such as a cyclic acetal, a substituent at position 8, and etherification of free phenolic groups. The last point was felt to be important by these authors, inasmuch as their findings indicated that ionization was possibly correlated with vitamin K activity.

ESTROGENIC ACTIVITY

A discussion of estrogenic activity in coumarin type compounds may be backgrounded advantageously by a brief consideration of the closely related isoflavones which, historically, were the first of the plant phenolics to show such activity. Indeed, the isoflavones may well be the source of coumarin estrogens (*e.g.*, coumestrol) in the plant as will become evident in the following discussion and from comments made in the consideration of biosynthesis in this paper. Estrus promoting substances in plants, however, have been known since 1926, shortly after the development of the Allen-Doisy test and are well reviewed by Bradbury and White (57) as well as Cheng, *et al.* (58). Continuing interest in naturally occurring estrogens has resulted recently in the isolation and characterization (59, 60) of miroestrol, virtually as active as diethylstilbestrol, from *Pueraria mirifica.*

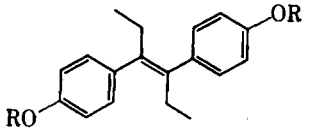
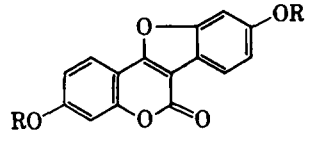
Recognition of the role of isoflavones as estrogens probably stems from the observations of Bennetts, *et al.* (61), in 1946 in connection with a serious sheep breeding problem encountered in western Australia. The problem of infertility was found to be caused by grazing on pastures of subterranean clover (*Trifolium subterraneum* L.). This finding was confirmed by Curnow, *et al.* (62), and was followed by the actual isolation of genistein (IVe),[1] an isoflavone, from this plant by Bradbury and White (63) and the suggestion by Biggers and Curnow (64) that this compound represented the principal estrogenic substance in the plant. Further studies have resulted in the demonstration of estrogenic activity in numerous other forage plants and the isolation

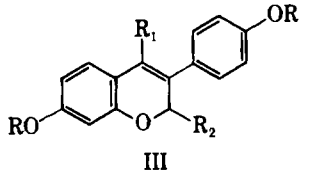
[1] Numbers such as IVe represent the structure to be found under that number in Table VI.

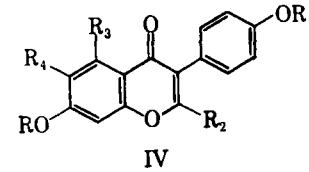
and synthesis of other interesting isoflavones such as formononetin (IVc), biochanin-A (IVf), and daidzein (IVd). The activities are well summarized by Cheng, *et al.* (58), and Micheli, *et al.* (65) (see Table VI).

The probable structural relationship of the isoflavones to diethylstilbestrol (Ia) did not escape the attention of workers in this field. Indeed, even a relationship with estradiol was visualized by some. The structure of coumestrol (IIa) may also be compared in this context. It was suggested by Biggers that genistein was probably a proestrogen, being metabolized in the animal body to the true estrogen. Thus, a number of compounds were prepared to explore the structure-activity relationships and to make compounds more closely resembling diethylstilbestrol. These studies are well summarized by Bradbury and White (57) and need not be discussed in detail here. Briefly, these studies led to the conclusion that, at best, the isoflavones were weakly estrogenic but that certain structural features seemed to promote activity in some measure. Among these features was the presence of a 5-hydroxy group that was suggested as being involved in hydrogen bonding with the carbonyl at position 4, presumably bringing about a greater contribution of the tautomeric enolic structure as against the ketonic structure. Coplanarity of the molecule as a whole was emphasized by Bradbury and White (66) in 1953 as being important to estrogenic activity. These workers also observed that reduction of the 2:3 double bond to form isoflavanones reduced activity. In contrast, it was noted (67) that isoflav-3-ens (III), as a group, were much more active than the isoflavones, possibly because of the much greater similarity to the diethylstilbestrol molecule. In this connection, it was noted that a 2- or 4-alkyl (or better, aryl) substituent conferred unusual activity on the isoflav-3-ens, whereas a methoxyl group at position 5 dropped activity. The isoflav-3-ens undoubtedly function as diethylstilbestrol does because structural modifications in both groups seem to affect activity similarly.

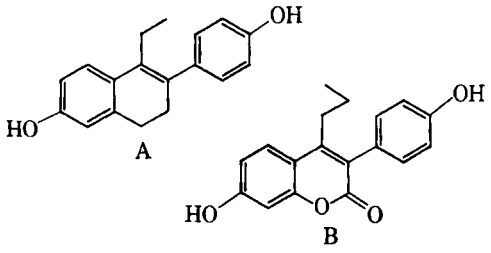
TABLE VI.—COMPARATIVE ESTROGENIC POTENCIES[a]

Compd. and Structure, Type	No.	Substituents	Dose Level, mg.[b]
I. Diethylstilbestrol derivatives		R	
 I	(*a*)	H—	0.00008
	(*b*)	CH_3CO —	0.00010
	(*c*)	$CH_3(CH_2)_{14}CO$—	0.00021
	(*d*)	CH_3—	0.00045
II. Coumestrol derivatives		R	
 II	(*a*)	H—	0.25
	(*b*)	CH_3CO—	0.33

III. Related isoflav-3-ens		R	R_1	R_2	
 III	(*a*)	CH_3CO—	C_2H_5—	CH_3—	0.00014
	(*b*)	CH_3—	C_2H_5—	CH_3—	0.00038
	(*c*)	CH_3CO—	H—	CH_3—	0.031
	(*d*)	CH_3—	H—	CH_3—	0.051
	(*e*)	CH_3—	C_6H_5—	H—	0.005
	(*f*)	CH_3CO—	C_2H_5—	H—	0.012
	(*g*)	CH_3—	C_2H_5—	H—	0.060
	(*h*)	CH_3—	CH_3 —	H—	0.208

IV. Related isoflavones		R	R_1	R_2	R_3	R_4	
 IV	(*a*)	H—	H—	CH_3—	H—	H—	21
	(*b*)	CH_3CO—	CH_3CO—	CH_3—	H—	H—	...
	(*c*)	H—	CH_3—	H—	H—	H—	31
	(*d*)	H—	H—	H —	H—	H—	11
	(*e*)	H—	H—	H—	HO—	H—	8
	(*f*)	H—	CH_3—	H—	HO—	H—	18
	(*g*)	H—	CH_3—	H—	H—	CH_3O—	(15)[c]

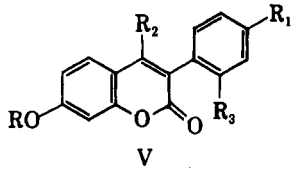
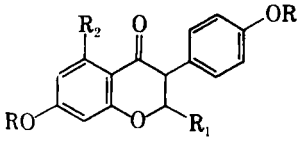
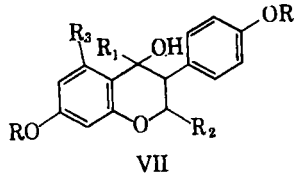
The matter of estrogenic activity of plant phenolics stood at this point until 1957 when Bickoff, *et al.* (68, 69), working with ladino clover (*Trifolium repens*), isolated an estrogenic coumarin which they named coumestrol (IIa). They later synthesized it as well (70). Further study showed that the same compound was the predominant one in ladino clover, strawberry clover (*Trifolium fragiferum*), and alfalfa (*Medicago sativa*). Comparison of estrogenic potencies showed that coumestrol was approximately 30 times as potent as genistein, although still far short of diethylstilbestrol. Interestingly enough, prior to the isolation of coumestrol, Mentzer, *et al.* (71, 72), as early as 1946, had anticipated the estrogenic activity of coumarins on purely theoretical grounds. These workers (72) based their researches on several diverse comparisons of structurally analogous compounds exhibiting similar activities and proposed that estrogenic activity should be exhibited by coumarins structurally related to diethylstilbestrol, but more particularly by preparing compounds directly related to 1-ethyl-2-*p*-hydroxyphenyl-3,4-dihydro-6-hydroxynaphthalene (A) which had been prepared by Dodds, *et al.* (73). Compound A was estrogenic in a dose of 100 mcg. and, essentially, Mentzer's modification was the replacement of $—CH_2CH_2—$ in A with the —COO— group. The most active compound in their series proved to be 3-(*p*-hydroxyphenyl)-4-*n*-propyl-7-hydroxycoumarin (B). Bickoff, *et al.* (68), compared this compound



with coumestrol and found the same order of activity (compare also IIa and Vj in Table VI).

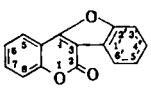
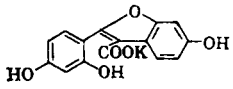
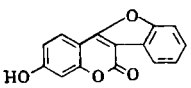
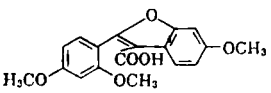
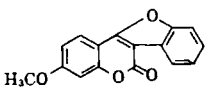
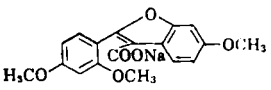
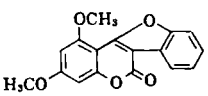
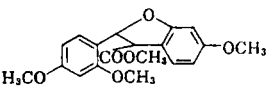
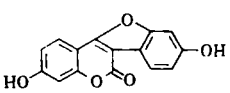
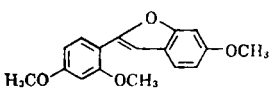
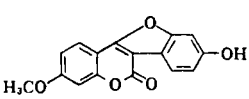
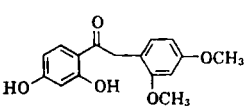
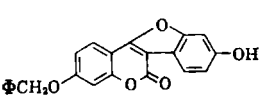
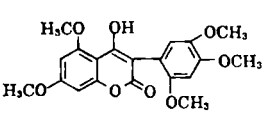
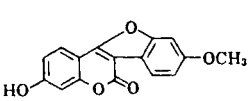
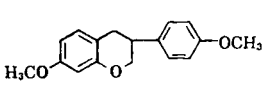
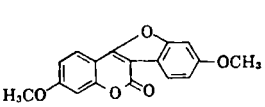
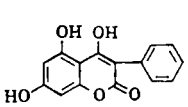
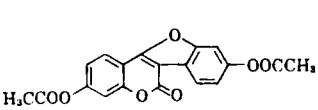
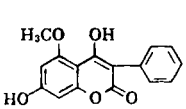
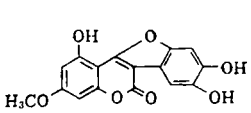
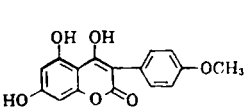
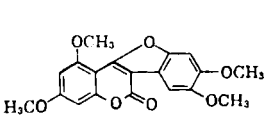
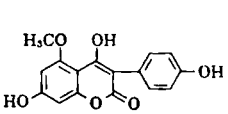
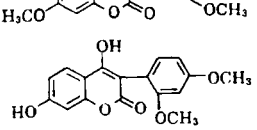
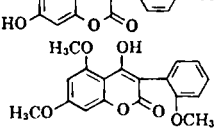
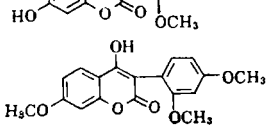
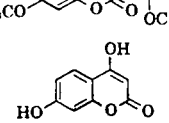
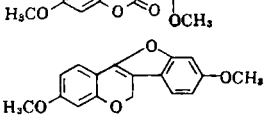
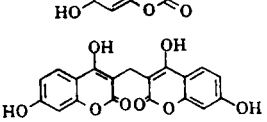
In a further study of structure-activity relationships, Bickoff, *et al.* (74), compared cou-

TABLE VI.—(continued)

Compd. and Structure, Type	No.	Substituents				Dose Level, mg.[b]
		R	R_1	R_2	R_3	
V. Related coumarins						
 V	(*a*)	H—	HO—	H—	H—	37
	(*b*)	CH_3CO—	CH_3COO—	H—	H—	40
	(*c*)	CH_3—	CH_3O—	H—	H—	(50)
	(*d*)	CH_3CO—	CH_3COO—	CH_3COO—	H—	(50)
	(*e*)	H—	HO—	HO—	H—	(40)
	(*f*)	H—	CH_3O—	HO—	H—	(50)
	(*g*)	H—	CH_3O—	HO—	CH_3O—	(25)
	(*h*)	H—	CH_3O—	*n*-C_3H_7—	H—	0.031
	(*i*)	CH_3—	CH_3O—	*n*-C_3H_7—	H—	0.032
	(*j*)	H—	HO—	*n*-C_3H_7—	H—	0.10
	(*k*)	H—	HO—	C_2H_5—	H—	(0.10)[d]
	(*l*)	H—	H—	C_2H_5—	H—	0.
	(*m*)	H—	CH_3O—	CH_3—	H—	0.2
VI. Related isoflavanones		R	R_1	R_2		
 VI	(*a*)	CH_3CO—	H—	H—		25
	(*b*)	H—	H—	H—		..
	(*c*)	CH_3CO—	CH_3—	H—		..
	(*d*)	H—	CH_3—	H—		..
	(*e*)	CH_3CO—	H—	CH_3COO—		18
VII. Related isoflavan-4-ols		R	R_1	R_2	R_3	
 VII	(*a*)	CH_3—	C_6H_5—	H—	H—	0.0035
	(*b*)	CH_3CO—	H—	CH_3—	H—	0.320

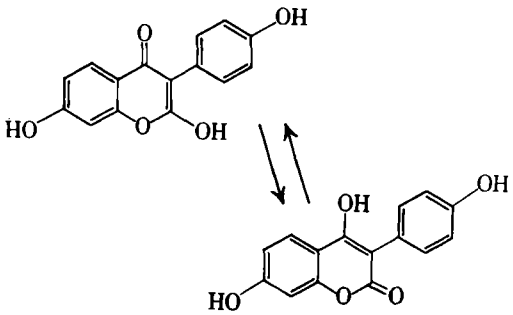
[a] This table is adapted from the tabular material given by Micheli, R. A., Booth, A. N., Livingston, A. L., and Bickoff, E. M., *J. Med. Pharm. Chem.*, **5**, 321(1962). [b] Represents the quantity of material fed to produce a 25-mg. uterine response compared to a uterine weight of 10 mg. for untreated animals. [c] Dose level in parentheses means that the compound was inactive at the indicated level. [d] Only slightly active at this level; higher dosage levels not tested because of lack of material.

TABLE VII.—RELATIVE ESTROGENIC ACTIVITIES OF COUMESTROL AND RELATED COMPOUNDS

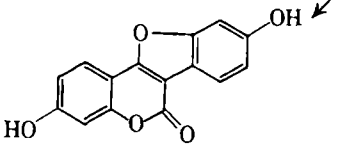
Compd. No.	Structural Formula	Relative Activity	Compd. No.	Structural Formula	Relative Activity
1		0	16		754
2		152	17		20
3		40	18		15
4		0	19		26
5		1000	20		3
6		540	21		16
7		189	22		0
8		150	23		0
9		359	24		0
10		850	25		0
11		1	26		3
12		0	27		0
13		22	28		0
14		0	29		0
15		0	30		0

mestrol with numerous structurally related compounds and obtained the results in Table VII. A number of interesting comparisons were made from a study of the results, although in no case was greater activity obtained than that of coumestrol itself. From the tabulated results it was observed that the phenolic hydroxyl groups in positions 4′ and 7 were quite important, inasmuch as their absence (compound 1, Table VII) led to inactivity. Some return of activity was noted with the presence of the hydroxyl at position 7 (compound 2, Table VII). Etherification of the phenolic groups (compounds 3 and 9, Table VII) results in reduced activity, a finding that is in conformity with almost all phenolic estrogens, except for the anomalous case of the 4-alkylcoumarins (65) where the reverse is true. It may be observed that etherification of the 4′-hydroxyl (compound 8, Table VII) reduces activity more than similar treatment of the 7-hydroxyl (compound 6, Table VII). Other nuclear substitution (*i.e.*, OH or OCH_3) as in the 3′ and 5 positions (compounds 11–14, Table VII) almost eliminates activity. Acetylation of the hydroxyl groups of coumestrol (compound 10, Table VII) results in very little loss in activity, possibly due to facile hydrolysis of these groups *in vivo* to regenerate coumestrol. The ether link bridging the 4 and 2′ positions appears to contribute substantially to activity since the lack of the ether bridge (compound 23, Table VII) produces a virtually inactive compound. Compounds 13 and 14 exhibit similar activity losses by opening of the 4:2′ ether bridge, although in this case the compounds bear a hydroxyl in position 4. Lack of activity in 4-hydroxycoumarins is general and is attributed to destabilization of the 3:4 double bond and, therefore, of the stilbene system which is now free to exist in tautomeric keto-enol forms. It has been suggested that the greater activity of coumestrol when compared with the isoflavones may be due to the principally ketonic character of the isoflavone at position 4. Reduction of the double bond at the 3:4 position (compounds 15, 23, Table VII) brings about loss of activity even though the ether linkage is still present in compound 15 (the C=O at position 2, however, is replaced by $—CH_2—$). Among the interesting observations made was that opening of the lactone ring to form the potassium salt of the resulting *o*-hydroxycinnamic acid (compound 16, Table VII) did not decrease activity, whereas formation of the corresponding *o*-methoxycinnamic acid, its methyl ester, and its salt (compounds 17–19, Table VII) virtually abolished activity. This difference in activity is undoubtedly because the potassium salt can readily revert back to coumestrol in the acid gastric environment, whereas the *o*-methoxy compound is incapable of doing so.

It has been suggested by Whalley (75) in a review of estrogens by Biggers that coumestrol derives its estrogenic activity by virtue of its stilbene-like structure which is analogous to that of diethylstilbestrol and that, furthermore, it bears a close structural relationship to diethylstilbestrol and estradiol. Structural alterations that disturb this stilbene-like structure would, therefore, reduce the similarity as has been suggested in the case of the 4-hydroxycoumarins with consequent lessening of estrogenic potency. Bate-Smith, quoted by Biggers (75), also suggests the possibility that coumestrol may be related to the isoflavones by postulating the conversion of an isoflavanol to coumestrol as in



Isoflavanol corresponding to Daidzein



Coumestrol

That the above speculation had a solid foundation is borne out by the findings of Grisebach and Barz (16).

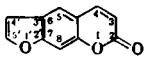
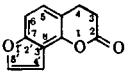
In conclusion, the reader's attention should be directed to the recent comprehensive study by Micheli, *et al.* (65), in which the authors examine the question of estrogenic structure-activity relationships of coumestrol, plant phenolics, and synthetic estrogens on a comprehensive and comparable basis. The findings of this group are summarized in Table VI adapted from their paper. Although their findings are ex-

tremely interesting, the full consideration of the paper is not within the scope of this review; the interested reader is urged to consult the original paper for details.

DERMAL PHOTOSENSITIZING ACTIVTY

The role of certain plant juices and extractives as dermal photosensitizing agents has been known for many years. Among the plants

TABLE VIII.—QUALITATIVE TESTS ON HUMAN SKIN IRRADIATED WITH LONG-WAVE ULTRAVIOLET LIGHT (3655 Å.)[a]

No.	Name		Activity[b]
		NATURAL FURANOCOUMARINS	
1.	Psoralen		++++
2.	Xanthotoxin	(8-Methoxypsoralen)	+++
3.	Bergapten	(5-Methoxypsoralen)	+++
4.	Angelicin		+
5.	Isobergapten	(5-Methoxyangelicin)	+
6.	Oxypeucedanin	[5-(β,γ-Oxido-isoamyloxy)-psoralen]	Sunlight only
7.	Xanthotoxol	(8-Hydroxypsoralen)	−
8.	Imperatorin	(8-Isoamyleneoxypsoralen)	−
9.	Bergaptol	(5-Hydroxypsoralen)	−
10.	Isopimpinellin	(5,8-Dimethoxypsoralen)	−
11.	Ostruthol	[5(β,γ-Dihydroxy-isoamyloxy)-psoralen angelic acid monoester]	−
		SYNTHETIC FURANOCOUMARINS	
	Related to Psoralen		
12.	4′,5′-Dihydropsoralen		−
13.	3,4-Dihydropsoralen		−
14.	4′-Methylpsoralen		+++
15.	4,4′-Dimethylpsoralen		+++
16.	4′-Phenyl-4-methylpsoralen		−
17.	Dimer of psoralen		−
18.	Thiopsoralen		−
	Related to Xanthotoxin		
19.	3,4-Dihydroxanthotoxin		−
20.	3-Methylxanthotoxin		+
21.	4-Methylxanthotoxin		+++
22.	4′-Methylxanthotoxin		++
23.	4′,3-Dimethylxanthotoxin		+
24.	4′,4-Dimethylxanthotoxin		+
25.	5′,4-Dimethylxanthotoxin		+++
26.	5′-Phenyl-4-methylxanthotoxin		−
27.	5-Chloro-xanthotoxin		+
28.	5-Nitro-xanthotoxin		−
29.	5-Amino-xanthotoxin		−
30.	5-Acetylamino-xanthotoxin		−
31.	8-Benzyloxypsoralen		+
32.	Thioxanthotoxin		−
	Related to Bergapten		
33.	4′,5′-Dihydrobergapten		−
34.	4-Methylbergapten		+
35.	4-Methyl-4′,5′-dihydrobergapten		−
36.	Allobergapten[c]		+
37.	4′,5′-Dihydroallobergapten		−
38.	4′,5′,3,4-Tetrahydroallobergapten		−
39.	4-Methylallobergapten		+
40.	4-Methyl-4′,5′-dihydroallobergapten		−
41.	5-Ethoxypsoralen		++
42.	5-Isopropoxypsoralen		+
43.	5-*n*-Propoxypsoralen		− (Sunlight +)
44.	5-*n*-Butoxypsoralen		− (Sunlight +)
45.	5-Isoamyloxypsoralen		− (Sunlight +)
46.	Psoralen-5-oxyacetic acid ethyl ester		−
47.	5-Benzyloxypsoralen		−
48.	8-Nitrobergapten		−
49.	8-Aminobergapten		−
50.	8-Acetylaminobergapten		−
51.	Bergapten-8-carboxylic acid methyl ester		+
52.	4′,5′-Dihydrobergapten-8-carboxylic acid methyl ester		−
53.	Bergapten-8-carboxylic acid		−
54.	4′,5′-Dihydrobergapten-8-carboxylic acid		−
55.	Dimer of bergapten		−
	Related to Angelicin		
56.	4-Methylangelicin		+
57.	Dimer of angelicin		−
	Related to Isopimpinellin		
58.	5,8-Dihydroxypsoralen		−
59.	Psoralenequinone		−

[a] Adapted from Musajo, L., and Rodighiero, G., *Experientia*, **18**, 153(1962). [b] ++++, Maximum activity; −, inactivity.
[c] Allobergapten has the structure

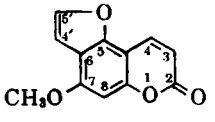


TABLE IX.—QUANTITATIVE TEST ON HUMAN SKIN[a,b]

Compd.	Minimum Length of Irradiation Needed for Outcome of Erythema, Min.	Relative Activity
Psoralen	6	100
4′-Methylpsoralen	10	60
Xanthotoxin	16	37.5
4,5′-Dimethylxanthotoxin	18	33.3
4,4′-Dimethylpsoralen	20	30
4-Methylxanthotoxin	20	30
Bergapten	22	27.5
5-Ethoxypsoralen	25	24
4′-Methylxanthotoxin	25	24
5-Isopropoxypsoralen	35	17.1
4-Methylbergapten	40	15
5-Chloroxanthotoxin	40	15
Angelicin	50	12
4′,4-Dimethylxanthotoxin	50	12
Allobergapten	50	12
4-Methylallobergapten	50	12
Bergapten-8-carboxylic acid methyl ester	50	12
3,4′-Dimethylxanthotoxin	55	11
3-Methylxanthotoxin	60	10
Isobergapten	60	10
4-Methylangelicin	60	10
4-Benzyloxypsoralen	60	10

[a] Adapted from Musajo, L., and Rodighiero, G., *Experientia*, **18**, 153(1962). [b] The substance is applied at a concentration of 5 mcg. per cm. and irradiated with a Philips HPW 125 lamp (3655 Å.) at 15 cm. from the skin.

involved are some of the common ones such as parsley, celery, figs, and parsnip (76, 77). Juices of various parts of these plants, after contact with the skin and exposure to sunlight, cause areas of erythema, hyperpigmentation and, occasionally, vesiculation on the skin. In retrospect, it now appears that plants causing these effects all contain furanocoumarins as the active agents. Possibly one of the first observations implicating these compounds with photosensitizing activity was that of Jois, *et al.* (78), in 1933 in connection with the Ayurvedic treatment of leucoderma with seeds of *Psoralea corylifolia*. These workers actually isolated psoralene and characterized it as a furanocoumarin, but the clinicians apparently were unable to grasp the significance of the finding, and it remained for others to bring it to light years later. Kuske (79), in 1938, through a consideration of plant extracts and pure furanocoumarins, ascertained that the latter were the responsible agents in the dermal response. Following this, in 1947, Fahmy and Abu-Shady (80) isolated three crystalline principles from the seeds of the empirically used antileucodermic vegetable drug *Ammi majus* L., *viz.*, ammoidin, ammidin, and majudin which were soon shown (81) to be the well known xanthotoxin, imperatorin, and bergapten, respectively. The story of their investigation, proceeding from an unidentified crude drug to the isolation of active crystalline principles, is well told by the Egyptian dermatologist, El Mofty (82). The method finally developed for treatment of vitiliginous areas, utilizing the dermal photosensitizing properties of the furanocoumarins, was either to paint the areas externally with a solution or to administer the drug orally and, in either case, to expose the patient to sunlight or ultraviolet irradiation. In responsive patients, repigmentation of the vitiliginous areas follows either with or without previous vesiculation.

The discovery of this unique activity of the furanocoumarins coupled with their utility in the treatment of leucoderma stimulated much activity among researchers seeking to evaluate other naturally occurring furanocoumarins as well as related synthetic compounds for the structure-activity profile of this interesting chemical nucleus. It is not possible to cover all of the modifications that have been attempted, but certain obvious structural requirements have been defined. Especially active in this area have been Musajo and co-workers in Italy (83–85) and the American group at the University

TABLE X.—FURANOCOUMARINS TESTED ON GUINEA PIG SKIN[a,b,c]

Compd.	Activity[d]
4-Methylpsoralen	++++
5′-Methylpsoralen	++++
5′,8-Dimethylpsoralen	++++
4,4′-Dimethylpsoralen	+
4,5′,8-Trimethylpsoralen	++++
3,4,5′,8-Tetramethylpsoralen	+
8-*n*-Propyl-4,5′-dimethylpsoralen	++
3-*n*-Butyl-4,5′,8-trimethylpsoralen	+
8-Acetyl-4,5′-dimethylpsoralen	++
8-Bromo-4,5′-dimethylpsoralen	++
8-Acetamido-4,5′-dimethylpsoralen	+
8-Acetyl-4,5′-dimethylpsoralen semicarbazone	+
3,4-Benzo-5′,8-dimethylpsoralen	+
3,4-Cyclohexeno-5,8-dimethylpsoralen	+
Anhydromarmesin	+
Marmesin	+
5′-Methylangelicin	+
3-Bromo-4′,5′-dihydroxanthotoxin	±
8-Amino-4,5′-dimethylpsoralen	−
4′,5′-Dihydroxanthotoxin	−
4′,5′-Dihydro-4-methylpsoralen	−
3,5-Dibromo-4′,5′-dihydro-8-methoxypsoralen	−
5-Bromo-8-methoxypsoralen	−
4′,5′-Dimethylangelicin	−

[a] Adapted from Musajo, L., and Rodighiero, G., *Experientia*, **18**, 153(1962). [b] Based on the work of Pathak, M. A., Fellman, J. H., and Kaufman, K. D., *J. Invest. Dermatol.*, **35**, 165(1960). [c] The administered amounts varied up to 1000 mcg. per cm.²; a 250-w. lamp, emitting U.V. radiations with $\lambda > 3200$ Å. was used and irradiation time was 45 minutes; distance 12–15 cm. [d] ++++, Maximum activity; −, inactivity.

of Oregon, among whom the active contributors have been Pathak, Fowlks, Fitzpatrick, etc. (86, 87). Musajo and Rodighiero (76) have reviewed the field recently, although the interested reader will wish to consult the proceedings of the 1959 symposium on psoralens and radiant energy as well (88). Members of the American group have been interested especially in the more fundamental aspects of photodynamic action, such as the biochemistry of melanin formation (89, 90), the mechanism of the photodynamic effect (91), the inhibition of enzyme systems (92), and the sensitization of bacteria (93, 94) by the furanocoumarins. The Italian group also has been active recently in trying to determine the mechanism of activity of these compounds (95).

Musajo and Rodighiero (76) have tabulated the relative qualitative (Table VIII) and quantitative (Table IX) activities of the naturally occurring coumarins and the synthetic congeners related to the natural prototypes. Table X summarizes the structure-activity relationships developed by Pathak, *et al.* (87). A consideration of the data offered in these tables indicates that maximum photosensitizing activity lies in the parent compound, psoralene, and that the various structurally related compounds have more or less reduced activity, depending on the ring system and nature of substituents. Significant activity requires a linear unreduced furanocoumarin ring system. Free phenolic groups inactivate the molecule, but the methyl ethers of the two possible phenols are both active. However, the dimethyl ether of the diphenol is inactive. Etherifying groups larger than methyl result in progressive reduction of activity as the size of the group increases. Nuclear substitution with methyl groups can cause significant retention of activity or loss depending on the position of the group. Thus, a methyl at the 4, 4′, 5′, or 8 positions may or may not inhibit activity, but a methyl at the 3 position invariably does so. Little success has been noted with the introduction of nitro, amino, or acetylamino groups. A unique ring modification in the form of the oxazolocoumarins (96) also leads to inactivity.

Fowlks (91) has analyzed the available evidence in an effort to determine the mechanism of the photodynamic effect of these compounds as well as of the multitude of structurally different but similarly active compounds. He feels that the primary event of photosensitization is the absorption of light by the photosensitizer molecule or by a complex of the photosensitizer with protein or nucleic acid. Following this, the events can become rather complicated, but it appears probable that an activated photosensitizer molecule can cause one of three results: (*a*) chemical combination of photosensitizer with a sensitive cell constituent, (*b*) chemical reaction, *i.e.*, oxidation of a sensitive cell constituent or, (*c*) indirect excitation of cell constituents which, through chemical changes, cause inactivities of vital cellular activities. He further suggests that there may well be a balance between damage suffered by the cell and its ability to repair the damage. Nonrepairable damage could lead to immediate or delayed death or to mutation. Indeed, mutation (97) is evident upon application of furanocoumarins to onion root tips, and 5-methoxypsoralen and psoralen are said to be almost as effective mutagenic agents as the most effective agent known, tryptaflavin. In connection with the observations of Fowlks, Pathak and Fellman (98) have noted that all biologically active furanocoumarins inducing photosensitization possess absorption and fluorescensce peaks of 320–360 and 420–460 mμ, respectively. Others, outside this range, are inactive. It also has been noted that the long wavelength ultraviolet lamp (3200 Å.) is needed to potentiate the response of the skin (erythema and sun-tanning) to the coumarins. It is thought that the skin response is associated in large measure with the capture of radiant energy of the proper wavelength (*i.e.*, 320–340 mμ) and that any changes in molecular shape, substitution pattern, etc., which disturb this specificity lead inevitably to decreased activity. Thus, the absorption of light of specific wavelength and the emergence of light of another wavelength in intimate contact with a sensitive cellular component is thought to be crucial to successful photosensitizing activity. A further observation may be made in that furanocoumarins have been quite definitely shown not to act by a photo-oxidative mechanism although other photosensitizing molecules such as the hematoporphyrins are known to act by way of this mechanism. Rodighiero and Capellina (99) have shown that, although furanocoumarins are dimerized under the influence of irradiation, the dimers are biologically inactive. The possibility that free radicals may be generated from excited furanocoumarin molecules under ultraviolet irradiation has been explored by Pathak, *et al.* (100), but, granting the biological changes that could occur in such an irradiated system, this probably does not adequately explain the marked activity of these compounds (76). Very recently, Musajo, *et al.* (95), have observed some interesting reactions of furanocoumarins with flavinmononucleotide (FMN). It appears that FMN will undergo reaction with furanocoumarins, but only with those that are photodynamically active and that

the reaction products appear to have been modified in the furan ring principally. Furthermore, they have demonstrated (albeit, in rather large doses) an antagonistic action of FMN toward the erythemal response expected from the psoralen-type molecule.

In spite of these findings, the precise mechanism whereby furanocoumarins function in the treatment of leucoderma is unknown. The biochemical events leading to the formation of the skin pigment, melanin, have been clarified, but no clearcut implication of the coumarins with it has been demonstrated. The formation of melanin is believed (89, 90, 101) to occur as depicted in Fig. 2. The coumarins apparently do not influence the tyrosine-tyrosinase reaction even under irradiation (76, 102, 103). A recent paper by Judis (104) indicates that xanthotoxin may cause the conversion of dihydroxyphenylalanine (DOPA) to melanin under the influence of ultraviolet light. Sen (101) finds himself tempted to look for another alternate additional pathway for melanin formation by using Robinson's scheme (105) for the formation of hydroxyindoles. Thus, it appears that the mechanism is uncertain and will have to await further clarification.

ANTIBACTERIAL ACTIVITY

Although coumarin itself has low antibacterial activity, other coumarins have been observed to have such activity in varying degrees. In particular, Dicumarol (106–108) has shown excellent activity against certain bacteria, *e.g.*, *Bacillus anthracis*, *Staphylococcus aureus*, *S. albus*, *Streptococcus pyogenes*, and *Pasteurella avicida*, although it has no growth suppressant action against some microorganisms. Cavallito (109) suggests that the predominantly Gram-positive activity of relatively lipophilic dicumarol is accounted for by the association of hydrophilic properties with Gram-negative activity and lipophilic character with Gram-positive activity. Ukita and co-workers (110) observed remarkable antibacterial activity against *Straphylococcus aureus* and *Mycobacterium tuberculosis* (avian type) by another (synthetic) coumarin, namely 3-acetyl-4-hydroxycoumarin. They point out that this compound is related to a number of other potent antibacterial compounds containing a tricarbonyl-methane unit, $[CH(-\overset{|}{C}=O)_3]$, as a common structural feature. Among the interesting noncoumarinic natural compounds containing this structural feature was the antibacterial lichen pigment, usnic acid. These workers also examined the effect of increasing the chain length of the 3-acyl group and found maximum activity in 3-*n*-decanoyl-4-hydroxycoumarin. Similar findings were reported by Toda, *et al.* (111). More recently, Ukita's group (112, 113) has studied the mode of action of the 3-

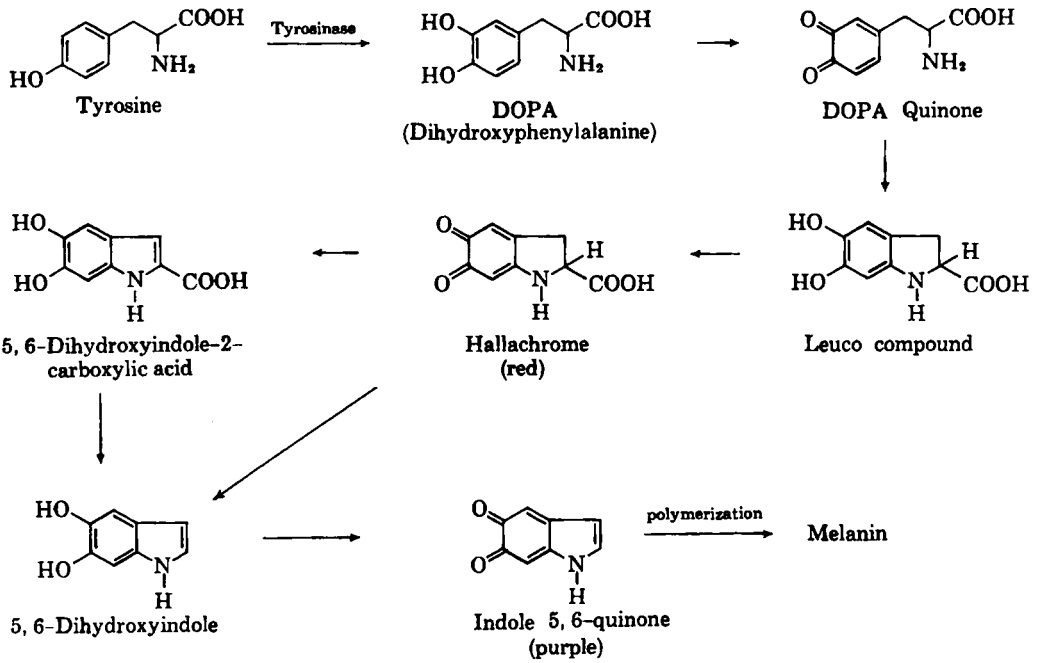


Fig. 2.—Enzymatic oxidation of tyrosine to melanin.

acetyl compound and concluded that it acts by an uncoupling effect on oxidative phosphorylation. It is interesting to note that Dicumarol is, likewise, a potent uncoupling agent (114). Indeed, Martius and Nitz-Litzow (115) were prompted to suggest that there was a direct relationship between the anticoagulant activity of Dicumarol and its congeners and the uncoupling effect, a hypothesis that since has become untenable.

Possibly the most important coumarin-type antibacterial agent is the antibiotic, novobiocin, isolated as a fungal metabolite of *Streptomyces niveus*. This unusual glycosidic coumarin has the distinction of being the first 4-hydroxycoumarin isolated as a fungal metabolite and is also the first to contain nitrogen as a part of the molecule. Its excellent antibacterial spectrum, chiefly against Gram-positive organisms and *Proteus vulgaris*, has led to its wide acceptance in medicine. It has also been found that it has a good action against *Leuconostoc mesenteroides*, the organism responsible for plugging of pipes in the sugar industry (116). Conversion to dihydronovobiocin resulted in a compound still retaining a wide spectrum of activity (116, 117). The aglycone derived from dihydronovobiocin by way of acid hydrolysis is also said to have activity (116), but the isoaglycone obtained by similar treatment of novobiocin is inactive. Recent patents (118, 119) have claimed good antibacterial activity for 3-amino-4,7-dihydroxy-8-methylcoumarin and the amide derivatives derived from novobiocin as well as for the esters of novobiocin which are claimed to have increased oil solubility and, thus, better properties for incorporation into oily ointment bases. The Japanese group of Okumura, *et al.* (120–122), has studied the compounds related to novobiocin in an effort to determine the structural features that contribute to its activity. Their first study described the synthesis of a number of 3-acylamino-4-hydroxycoumarin derivatives, several of which showed good antibacterial activity. One of the studies (121) undertaken was on 3-alkenyl-substituted 4-hydroxybenzoic acids related to the corresponding structural unit in novobiocin. The ethyl esters of some of these acids (*i.e.*, 3-(3-methyl-2-butenyl)-4-hydroxybenzoic acid and 3-allyl-4-hydroxybenzoic acid) exhibited antibacterial activity remindful of that shown by the well known *p*-hydroxybenzoates. In the final summation of their structure-activity studies (122) they pointed out that the presence of 3-(*O*-carbamoyl)-novobioside bonded at the 7 position of 3-(3-alkenyl-4-hydroxybenzamido)-4-hydroxycoumarin played an important role in the activity of novobiocin. Rodighiero, *et al.* (123), also have examined derivatives of 3-aminocoumarin for antibacterial activity and have found their best compound to be 3-amino-7,8-dihydroxycoumarin which was particularly effective against *Streptococcus pyogenes* and moderately effective against a variety of other bacteria.

Another coumarinic antibiotic that has been of interest is chartreusin, isolated as a metabolite from *Streptomyces chartreusis* (343). It is pre-

TABLE XI.—EFFECT ON FLOW RATE THROUGH ISOLATED RABBIT HEART OF VARIOUS PYRANOCOUMARINS

Compd.	Concn.	Rate of Flow—Control ml./2 min.	Rate of Flow—Sample, ml./2 min.	Increase, %	Relative Potency
Khellin	1:60,000	26.5	37	40	1
Visnagan	1:150,000	30.7	43	41	2.5
Khellin	1:30,000	28.0	39.7	41.8	1
Visnadin	1:60,000	21.0	49.1	131.6	...
Visnadin	1:300,000	28.5	39.7	39.2	10
Khellin	1:30,000	26.5	37.0	39.6	1
Visnadin mother liquors	1:240,000	23.5	30.8	31.2	Approx. 8
Khellin	1:15,000	24.7	45.6	84.6	1
Dihydrosamidin	1:75,000	24.7	45.0	82.2	5
Khellin	1:15,000	29.2	47.7	63.2	1
Papaverine·HCl	1:75,000	27.2	37.2	36.7	2.5
Papaverine·HCl[a]	1:205,000	23	41	78	...
Pteryxin	1:225,000	22	45	105	...
Papaverine·HCl	1:140,000	15	28	87	...
Pteryxin	1:185,000	16	37	131	...
Papaverine·HCl	1:140,000	12	28	133	...
Pteryxin	1:140,000	12	28	133	...
Papaverine·HCl	1:89,000	17	22	30	...
Pteryxin	1:176,000	23	44	91	...
Papaverine·HCl	1:112,000	14	28	100	...
Pteryxin	1:120,000	16	30	88	...

[a] This value and those following are taken from the Ph.D. dissertation of Robert E. Willette entitled "Structural Studies of Certain Pharmacologically Active Coumarins Isolated from Umbelliferae," University of Minnesota, Minneapolis, 1960, p. 56. The data were supplied by Hazleton Laboratories, Falls Church, Va. The same laboratory supplied the data in the first part of the table, these data having been reported in *J. Am. Chem Soc.*, **79**, 3538(1957).

dominantly active against Gram-positive organisms and mycobacteria (344) and also acts against *Micrococcus pyogenes* v. *aureus* phage. It exhibits possible cumulative toxicity and has not been exploited commercially. Work on the structure (345, 346) finally resulted in the elucidation of its structure by Simonitsch, *et al.* (347).

Tuberculostatic activity by coumarins has been noted in a few cases. Bersch and Döpp (124) examined this activity as related to *p*-aminosalicylic acid (PAS) and found that *trans*-*p*-aminocinnamic acid was one-fourth as active as PAS and that the analogous 7-amino-4-methylcoumarin was one-half as active as PAS. Rodighiero, *et al.* (125), working with furanocoumarins, found that only psoralen and xanthotoxin (coumarin less so) were able to inhibit growth of the tubercle bacillus significantly.

Antifungal activity has been noted, in passing, by many authors. One of the more recent studies is that of Chakraborty, *et al.* (126), who found that of 17 natural coumarins tested, the psoralenes, including psoralen and imperatorin, were the most effective antifungal agents tested.

VASODILATOR ACTIVITY

Although the coumarins are well regarded in the managment of symptomatic coronary arteriosclerosis, there is a growing opinion that holds the mechanism of activity to be vasodilatory rather than anticoagulant (127–131). One of the most recent studies in support of this hypothesis is that of Blake, *et al.* (131), who have shown, by a new plethysmographic technique, that the coronary vasodilator activity of dicumarol and warfarin on swine coronary arteries is comparable to the activity of aminophylline and nitroglycerin. The authors suggest that the vasodilator activity is brought about by a direct effect of the unchanged compound on the wall of the artery rather than by some indirect means (*e.g.*, by a metabolite or *via* the central nervous system). It was determined further that the concentrations of bishydroxycoumarin achieved in their experiments were approximately of the same order as those obtained in conventional anticoagulant therapy.

Other coumarins have been investigated also as coronary vasodilators following the original studies on *Ammi visnaga* L. which resulted in the marketing of khellin, a furanochromone, as a coronary vasodilator. The fact that certain impure preparations of khellin showed a better vasodilatory activity than the pure chromone in controlling angina of effort stimulated a search for the obviously more potent contaminant in the noncrystallized "visnagan" fraction of Samaan (132). This contaminant, originally described as "visnagan," was isolated in a crystalline state as the coumarin, visnadin, in 1952 by Smith, *et al.* (133). More recently, by carrying out more refined isolations (134) they obtained and structurally defined three strongly vasodilatory coumarins, *viz.*, visnadin, samidin, and dihydrosamidin. By studying the effect of these principles on the rate of flow through isolated rabbit heart, they obtained the results shown in Table XI. Willette and Soine, for the closely related coumarins pteryxin and suksdorfin (135), have shown a somewhat more potent action (see Table XI). Visnadin has been employed abroad for the treatment of angina pectoris (136, 137) with some success, although there is no U. S. product of comparable nature.

MOLLUSCACIDAL ACTIVITY

An interesting study by the Egyptian researchers, Schönberg and Latif (138), has demonstrated the possibility of snail control, at least in countries where furanocoumarin-containing plants can be grown with little expense. They have shown that bergapten and isopimpinellin (xanthotoxin less effectively) exert a molluscacidal activity comparable to some of the most powerful synthetic organic agents known, *e.g.*, dinitro-*o*-dicyclohexylphenol and sodium pentachlorophenate (Dow G) The objective, of course, is to control the snail *Biomphalaria boissi* which is known to be the intermediate aquatic host for *Schistosoma mansoni*, the blood fluke responsible for human schistosomiasis (*i.e.*, bilharziasis).

Utilizing snails obtained from their natural habitat as test objects under controlled conditions, they were able to show pronounced molluscacidal activity. Their preliminary observations were confirmed and extended by Evans and Zachary of the U. S. Naval Medical Research Unit No. 3, Cairo, and are summarized in Table XII. Xanthotoxin has not been included in the table because it showed no activity at 5 p.p.m. and only a 25% kill at 10 p.p.m. At 50 p.p.m.,

TABLE XII.—COMPARATIVE MOLLUSCACIDAL ACTIVITIES

Compd.	5 p.p.m.	2 p.p.m.
Bergapten	32/32 (100%)[a]	22/32 (69%)
Isopimpinellin	22/32 (69%)	3/32 (9%)
Dinitro-*o*-cyclohexylphenol	13/16 (81%)	14/32 (44%)
Sodium pentachlorophenate	10/16 (63%)	2/32 (6%)

[a] Percentage kill.

however, it gave a 100% kill. In the table the parentheses include the percentage kill. Schönberg and Latif have suggested that it might be possible to control snails economically by growing plants (*e.g.*, *Ammi majus*) containing the furanocoumarins, harvesting them, and simply throwing them into the channels infected with snails. As far as the author is aware, the suggestion has not been implemented.

ANTHELMINTIC ACTIVITY

It has long been known that certain naturally occurring lactones, *e.g.*, γ-butyrolactone, santonin, etc., possess anthelmintic properties. That such activity also was present in the coumarin lactones was demonstrated by Baldwin (139). Since then most of the interest in coumarins as anthelmintics has been among the Japanese. Ito, *et al.* (140, 141), found only weak activity in coumarin, 2-thiocoumarin, and 4-methylcoumarin, but Nakabayashi, *et al.* (142), working with *Ascaris suilla*, showed that methyl, hydroxy, and methoxyl derivatives of coumarin possessed significant activity and that the 7- and 3-methylcoumarins were particularly effective, although the presence of both 7-methyl and 3-alkyl substituents simultaneously depressed activity (143). Longer alkyl chains than methyl depressed activity likewise. Comparison of 3:4-dihydrocoumarin and its methyl derivatives with the corresponding unreduced compounds (144) indicated that there was no parallelism of action between the two groups and that the dihydro derivatives were uniformly less effective than the parent compounds. Ito and Kitagawa (145) found the same to be true when they compared the efficacies of coumarin, 2-thiocoumarin, and 4-methylcoumarin and their 3:4-dihydro derivatives. They noted that compounds melting between 70 and 100° all seemed to have good efficacy, and that those melting over 100° were uniformly inactive. These workers also studied the mode of action of these compounds against *Ascaris suilla* (146). Ishifuku, *et al.* (147), studied a number of umbelliferone and esculetin derivatives and found that the isopropyl ether of the former provided the greatest activity and that esculetin derivatives were, at best, only weakly active. They noted, too, that there was correlation between a low melting point and anthelmintic activity. Nakabayashi (148) showed that, in general, the several methylthiocoumarins were relatively less effective than the corresponding coumarins. He also examined the α- and β-naphthocoumarins and the 1- and 4-methyl as well as the tetrahydro-, hexahydro-, and dodecahydro derivatives (149). The most potent compound tested was 4-methyl-hexahydro-α-naphthocoumarin.

SEDATIVE AND HYPNOTIC ACTIVITY

Kreitmair (150) showed that coumarin possessed a definite hypnotic and sedative action on mice on oral administration but discounted any possible therapeutic application because of the high toxicity that made the margin of safety too small. The toxicity of coumarin has also been studied by Hazleton, *et al.* (151), who were unable to show really harmful effects but pointed out that longer term studies should be carried out. Ito, *et al.* (152), examined several coumarins for analgesic and hypnotic effects. They concluded that the intact 3:4 double bond was necessary for activity and noted that the potency was in the order of coumarin nucleus with α-pyrone ring *o*-condensed with naphthalene, the same condensed with benzene, and the benzene ring condensed to the 6 position. A surprisingly powerful effect was noted with ethyl coumarin-3-carboxylate alone among the ethyl coumarin carboxylates. They noted no great differences between α- and γ-pyrone ring systems. Ito, *et al.* (153), in 1953 again studied several different coumarins and found activity in coumarin-3-carboxylic acid diethylamide. Introduction of $-CH_2-$ between the coumarin ring and $-CON(C_2H_5)_2$ caused loss of activity as did conversion to the isomeric isocoumarin compound. They confirmed their earlier finding in this group of compounds that reduction of the 3:4 double bond was deleterious to activity. Kitagawa (154) observed that an alkyl group in the 3 position gave a hypnotic effect if the number of carbons was odd but imparted toxicity if even (peak with ethyl). The effect of the methyl group, in decreasing order of activity, was 8-, 3-, and 4-methyl. However, a significant finding was that the difference between the hypnotic and lethal dose was slight except with the 4- and 8-methyl compounds. Kitagawa (155) was able also to relate the hypnotic effect of coumarin derivatives to the suppressive action on the cytochrome level in conformity with the general proposal of Michaelis and Quastel (156) relating to hypnotics and anesthetics.

ANALGESIC ACTIVITY

In 1957, Stern, *et al.*, published a paper relating to the analgesic and antipyretic effects of vitamin K and dicumarol with special reference to 4-hydroxycoumarin (157) They made the startling statement that they were able to obtain

analgesic effects from 4-hydroxycoumarin with a minimum dose of 0.025 mcg./Kg. This proposal was examined by Adami (158) and Adami, *et al.* (159), in which they administered 4-hydroxycoumarin to albino rats in a well controlled study in doses of 0.010 to 250 mcg./Kg. orally, intraperitoneally, and subcutaneously. They found no analgesic activity of significance.

HYPOTHERMAL ACTIVITY

Kitagawa, *et al.* (160–162), examined a large group of coumarins for their ability to reduce body temperature. They concluded that most coumarins possess an effect of this kind. Among the many relationships noted they observed that methyl or propyl in the 3 position had a particularly strong action. Reduction of the 3:4 double bond reduced activity, but the introduction of a 2-thio group had little effect. A methyl group attached to the α-pyrone ring was preferable to having it attached to the benzene moiety of coumarin. With respect to 2-thiocoumarin they observed that there was a complete parallelism between the amount distributed in the brain and the hypothermal action.

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Research Articles

Quinazolines and 1,4-Benzodiazepines XVII

Synthesis of 1,3-Dihydro-5-pyridyl-2*H*-1,4-benzodiazepine Derivatives

By R. IAN FRYER, R. A. SCHMIDT, and L. H. STERNBACH

The synthesis of a series of 5-pyridyl analogs of the psychopharmacologically active class of compounds, 1,4-benzodiazepines, is described. These compounds are being screened for psychosedative, muscle relaxant, and anticonvulsant activity.

THE NEW PSYCHOTHERAPEUTIC agents of the 1,4-benzodiazepine class of compounds, chlordiazepoxide[1] and diazepam,[2] have received wide attention in recent years (1, 2). As a continuation of our earlier work on the synthesis of analogs of 1,4-benzodiazepines (3), we have prepared for pharmacological evaluation several derivatives of 1,3-dihydro-5-(2-pyridyl)-2*H*-1,4-benzodiazepin-2-one and 1,3-dihydro-5-(4-pyridyl)-2*H*-1,4-benzodiazepin-2-one.

The 2-aminobenzoylpyridines III*a,b* (H)[3] used as starting materials for these syntheses are described by Schofield and co-workers (4, 5). However, as recent work by Raynolds and Levine (6) makes 4-phenacyl pyridine readily available, we were able to prepare 4-(2-aminobenzoyl)pyridine III*b* (H) by the much easier route used by Schofield and Ockenden for the synthesis of 2-(2-aminobenzoyl)pyridine III*a* (H).[4] These workers utilized the oxidative fission of the 2,3-double bond of the appropriate indole I*a* (H), followed by hydrolysis of the 2-(2-benzamidobenzoyl)-pyridine II*a* (H) thus obtained.

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[1] Marketed as Librium by Hoffmann-La Roche, Inc., Nutley, N. J.

[2] Marketed as Valium by Hoffmann-La Roche, Inc., Nutley, N. J.

[3] III*a* (H) indicates compound III*a*, R = H (Scheme I); III*a* (Br) indicates compound III*a*, R = Br, etc.

[4] We found chromium trioxide preferable to ozone as an oxidant for *Ia, b* (H). For example, see Schofield, K., and Theobald, R. S., *J. Chem. Soc.*, **1949**, 796.