PHYSIOLOGICALLY ACTIVE UNSATURATED LACTONES

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THE occurrence of an unsaturated lactone ring in many physiologically active compounds has been commented upon by several writers,[1, 2, 3, 4], but no detailed survey of the activities shown by members of this group of compounds has yet appeared. Several papers published during the last five years have provided a certain amount of correlation in the various aspects of this field, and it seems desirable to gather together the available data.

The physiological activities shown by unsaturated lactones may be divided into the following six groups:

(I) Selective inhibition of the growth of animal tissues.
(II) Antibiotic activity.
(III) Inhibition of the germination of seeds and of plant growth.
(IV) Fish poison and insecticidal activity.
(V) Cardiac activity.
(VI) Miscellaneous, including anthelmintic and hæmorrhagic activity.

It seems likely that activity in any one of the first four of these groups implies the possession of some activity in any of the remaining three of the four, but how far these various types of activity may be considered to be differing responses—modified by substituents in some special cases, *e.g.*, the antibiotics—to the same basic stimulus is not known.

(I) *Selective Inhibition of the Growth of Animal Tissues*

The suggestion that an unsaturated lactone exerted a selective inhibitory effect on the growth of animal tissues was first made by P. B. Medawar, G. M. Robinson, and R. Robinson.[5] It had been shown by T. B. Heaton in 1926 [6] that there was present in boiled chick embryonic extract a factor which permitted the free growth *in vitro* of epithelial tissue but inhibited the growth of connective tissue (fibroblast, mesenchyme). Similarly, epithelial tissues grew very freely, whereas connective tissues did not grow at all, in malt extract.

The factor occurred in a number of adult mammalian organs, and Heaton suggested that the cessation in adults of connective tissue growth, as contrasted with the continued reproduction of epithelial tissue, was to be attributed to the controlling action of this factor. In a later paper,[7] Heaton examined further the physiological effects of this malt extract,

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[1] E. Ott, Houben-Weyl's "Methoden der Organischen Chemie", Liepzig, 1930, Vol. III, p. 612.

[2] H. Veldstra and E. Havinga, *Enzymologia*, 1944, **11**, 373.

[3] P. Läuger, H. Martin, and P. Müller, *Helv. Chim. Acta*, 1944, **27**, 892.

[4] A. E. Oxford, *Ann. Rev. Biochem.*, 1945, **14**, 749.

[5] *Nature*, 1943, **151**, 195.

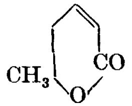
[6] *J. Path. Bact.*, 1926, **29**, 293.

[7] *Ibid.*, 1929, **32**, 565.

and showed that when injected into young mammals it caused an immediate arrest of growth, and when tested on adult rats implanted with the Jensen rat sarcoma, the growth of the tumour was arrested.

Heaton's results have since been confirmed by other workers.[8, 9, 10] P. B. Medawar showed that the action of the malt-extract factor on explants of heart, spleen, buffy coat, periosteum, perichondrium, and Jensen rat sarcoma indicated that the activity extended to all mesenchyme derivatives, and further showed that the inhibitor could be recovered from wheat, maize, oats, barley, wholemeal flour, baked wholemeal bread, and oranges. (It should be noted that the assumption that selective inhibitors obtained from all these sources were identical was, of necessity, based solely on the similarity of their action.)

Medawar, Robinson, and Robinson [5] concentrated the differential inhibitor present in malt by vigorous steam distillation of malt extract, and found that its properties " indicated the probability that it was an unsaturated lactone $C_6H_8O_2$ ". Medawar *et al.* pointed out that a naturally occurring unsaturated lactone of this empirical formula, " parasorbic acid ",[11] was already known and had been shown to be D-δ-hex-α-enolactone (I).[12, 13]


(I.)

Racemic δ-hex-α-enolactone was synthesised in small yield by condensation of aldol with malonic ester in pyridine solution (the main product being sorbic acid).

$$\begin{tikzcd}
CH_3{\cdot}CH(OH){\cdot}CH_2{\cdot}CHO + CH_2(CO_2Et)_2 \arrow[r] & CH_3{\cdot}CH(OH){\cdot}CH_2{\cdot}CH{=}C(CO_2Et)_2 \arrow[dl] \arrow[d, "\text{hydrolysis and decarboxylation}"] \\
CH_3{\cdot}CH{=}CH{\cdot}CH{=}CH{\cdot}CO_2H & (I)
\end{tikzcd}$$

The synthetic product was apparently not obtained in sufficient quantity to be examined in detail, but its properties were found to resemble those of the malt distillate lactone ; in particular, it exhibited the differential growth-inhibiting property, although it was somewhat less active than the natural factor. Synthetic DL-hexenolactone prepared by semihydrogenation of the acetylenic hydroxy-acid obtained by carboxylation of the ethynylcarbinol derived from propylene oxide was obtained in better yield and adequately purified,[14, 15] but the activity was feeble compared with that of later natural extracts prepared at Oxford.[16]

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[8] A. H. Drew, *Brit. J. Exp. Path.*, 1927, **8,** 176.

[9] A. M. Brues, E. B. Jackson, and J. C. Aub, *Proc. Soc. Exp. Biol. Med.*, 1936, **34,** 270.

[10] P. B. Medawar, *Quart. J. Exp. Physiol.*, 1937, **27,** 147.

[11] A. W. Hofmann, *Annalen*, 1859, **110,** 129.

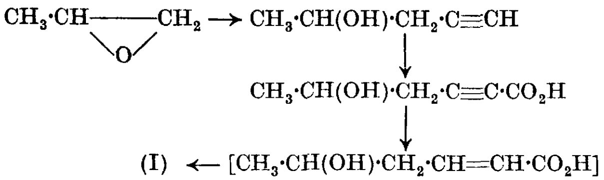
[12] O. Doebner, *Ber.*, 1894, **27,** 345.

[13] R. Kuhn and D. Jerchel, *ibid.*, 1943, **76,** 410.

[14] L. J. Haynes and E. R. H. Jones, *Nature*, 1945, **155,** 730.

[15] *Idem, J.*, 1946, 503.

[16] Sir R. Robinson and Dr. P. B. Medawar, private communication.



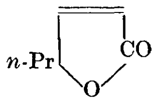
$$CH_3{\cdot}CH\underset{O}{\diagdown\!\diagup}CH_2 \rightarrow CH_3{\cdot}CH(OH){\cdot}CH_2{\cdot}C{\equiv}CH \rightarrow CH_3{\cdot}CH(OH){\cdot}CH_2{\cdot}C{\equiv}C{\cdot}CO_2H \rightarrow [CH_3{\cdot}CH(OH){\cdot}CH_2{\cdot}CH{=}CH{\cdot}CO_2H] \rightarrow (I)$$

R. Kuhn, D. Jerchel, F. Moewus, E. F. Möller, and H. Lettré [17] have confirmed the inhibitory action of a synthetic DL-δ-hexenolactone prepared by treatment with water of the dibromohexoic acid formed by addition of hydrogen bromide to sorbic acid :

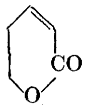
$$CH_3{\cdot}CH{=}CH{\cdot}CH{=}CH{\cdot}CO_2H \rightarrow CH_3{\cdot}CHBr{\cdot}CH_2{\cdot}CHBr{\cdot}CH_2{\cdot}CO_2H \rightarrow (I)$$

and have shown that the natural (optically active) parasorbic acid shows an activity of the same order ; hence the activity is apparently independent of stereochemical configuration.

Nevertheless, although δ-hexenolactone and the natural inhibitory factor are not identical, the fact that a relatively simple compound shows this differential activity to some extent is of interest. The activity is not common to all αβ-unsaturated lactones ; of several αβ-unsaturated γ-lactones,[18] γ-heptenolactone (II) showed a very slight differential activity, and the others were inactive. On the other hand, δ-pentenolactone (III) was about twice as active as δ-hexenolactone.[16]



(II.)



(III.)

The selective growth-inhibitory action of synthetic δ-hexenolactone has been studied in some detail by T. S. Hauschka,[19] who found that the flatworm *Dugesia tigrina*, on being cultured in an M/20,000-solution of δ-hexenolactone, developed a three-branched lesion in the dorsal wall corresponding in pattern to the triclad intestine. Measurements on the lesion area, survival, and extent of healing permitted a quantitative comparison between tests and controls. The activity was tissue-differential, connective tissue being most, and epithelial tissue least, affected. Hauschka found that the activity could not be duplicated by a large variety of inorganic and organic substances.

It was suggested by Medawar *et al.*[5] that the differential action produced by δ-hexenolactone was due to its ability to interfere in some way with a growth factor, possibly pantothenic acid, which was more specifically required by fibroblasts than by epithelial cells. It was shown, however, by Kuhn *et al.*[17] that addition of excess of pantothenic acid did not interfere with the inhibition of the growth of chick fibroblasts. These results were confirmed in

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[17] *Naturwiss.*, 1943, **31**, 468.

[18] L. J. Haynes and E. R. H. Jones, *J.*, 1946, 954.

[19] *Nature*, 1944, **154**, 769.

the flat-worm tests by Hauschka, who tested the behaviour of the flat-worm in media containing α-alanine, β-alanine, glycine, *iso*leucine, D-glutamic acid, or glutathione, as well as M/20,000-hexenolactone. Both α- and β-alanine and glutathione (the cysteine component of which is actually thioalanine) had a specific protective influence. Glycine, *iso*leucine, and D-glutamic acid did not counteract the inhibitor but actually added to its activity.

The protective nature of the alanines was apparently confined to an optimum concentration within narrow limits, since they ceased to give protection against M/20,000-hexenolactone at concentrations *above* M/5000 ; M/1000-α- and -β-alanine as such had no visible toxic effect. Hauschka therefore suggested that the differential growth-inhibitory property exhibited by δ-hexenolactone involved a specific alanine mechanism distinct from the alanine link in the biosynthesis of pantothenic acid.

In a later paper, T. S. Hauschka, G. Toennies, and A. R. Swain [20] showed that cysteine (but not cystine) was antagonistic to δ-hexenolactone, and established by both spectrophotometric and colorimetric methods that direct and reversible reaction took place between the lactone and the thiol grouping. Cysteine counteracted the biological effect of δ-hexenolactone by a mechanism distinct from that of inactivation by alanine, and hence Hauschka *et al.* were led to suggest that δ-hexenolactone might exert its effect on cellular proliferation mainly through its reactivity with SH groups essential to enzyme function.[cf. 21, 22, 25]

Clearly, much work remains to be done in this field. The significance of the suggestions concerning the mode of action of hexenolactone has yet to be determined ; since there is a marked structural specificity in the growth-inhibitory activity shown by αβ-unsaturated lactones, it hardly seems likely that the simple theory of reactivity with SH groups, which is common to most, if not all, αβ-unsaturated lactones,[cf. 23, 24] will give a complete explanation. Further work on the natural growth-inhibitory factor, shown by Medawar *et al.* to possess the properties of an unsaturated lactone, is also needed.

(II) *Antibiotic Activity*

The simple αβ-unsaturated lactones possess very weak antibacterial activity. For example, α- (IV) and β-angelicalactones (V) inhibit the growth of *Escherichia coli* at a concentration of 1 in 1000,[26] and the growth of *Staphylococcus aureus* is inhibited by δ-hexenolactone (I) (both the natural dextrorotatory compound and the synthetic racemate) and by γ-hexenolactone (VI) at 1 in 2000.[17] Kuhn *et al.*[17] have also shown that among

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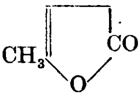
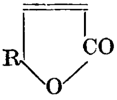
[20] *Science*, 1945, **101,** 383.
[21] Bersin, *Ergebn. Enzymforschung*, 1935, **4,** 68.
[22] E. S. G. Barron and T. P. Singer, *Science*, 1943, **97,** 356.
[23] W. B. Geiger and J. E. Conn, *J. Amer. Chem. Soc.*, 1945, **67,** 112.
[24] C. J. Cavallito and T. H. Haskell, *ibid.*, p. 1991.
[25] C. J. Cavallito and J. H. Bailey, *Science*, 1944, **100,** 390.
[26] H. Baer, M. Holden, and B. C. Seegal, *J. Biol. Chem.*, 1946, **162,** 65.

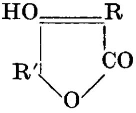
the bacteria are some (lactic acid bacteria, *Streptobacterium plantarum*) which like animal epithelia (cf. Section I) are relatively unresponsive.



(V.)



(IV.), R = CH_3
(VI.), R = C_2H_5



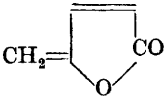
(VII.)

Fission of the lactone ring with alkali results in loss of antibacterial activity as does hydrogenation of the double bond.[26]

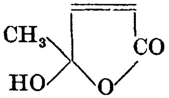
Several derivatives of tetronic acid of the type (VII) isolated from mould metabolic products by Raistrick and his co-workers [27] have been reported to show no antibacterial activity, but it is possible that concentrations of the order 1 in 1000 were not tested.

The antibacterial activities of the simple compounds are only of academic interest, but some substituted $\alpha\beta$-unsaturated lactones, especially those with an unsaturated substituting group, show a fairly high degree of antibacterial activity which makes desirable their discussion in some detail.

Protoanemonin.—Probably the simplest unsaturated lactone showing any marked antibacterial activity is protoanemonin (VIII), the γ-lactone of β-acetylacrylic acid. This substance, a pale-yellow vesicant oil, was first isolated by Y. Asahina and A. Fujita [28] by steam distillation of *Ranunculus japonicus* and has since been found [26] to be responsible for the antibacterial activity of extracts of *Anemone pulsatilla*.[29, 30]



(VIII.)



(IX.)

Three syntheses of protoanemonin have been reported,[28, 31, 32] of which the most satisfactory is that due to E. Shaw, in which acetylacrylic acid is cyclised by the acid-catalysed action of acetic anhydride.

$$CH_3{\cdot}CO{\cdot}CH{=}CH{\cdot}CO_2H \xrightarrow{H_2SO_4-Ac_2O} \text{(VIII)}$$

On the basis of spectroscopic evidence, Shaw considers that β-acetylacrylic acid exists as the lactone (IX), and suggests that the apparent cyclisation is merely a straightforward dehydration. Baer *et al.*[26] state that β-acetylacrylic acid shows no antibacterial activity; on the other hand,

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[27] P. W. Clutterbuck, W. N. Haworth, H. Raistrick, G. Smith, and M. Stacey, *Biochem. J.*, 1934, **28,** 94; P. W. Clutterbuck, H. Raistrick, and F. Reuter, *ibid.*, 1935, **29,** 300, 871, 1300; see also H. Raistrick, *Ann. Rev. Biochem.*, 1940, **9,** 571.

[28] *Acta Phytochim. Japan*, 1922, **1,** 1; *Chem. Zentr.*, 1922, III, 712.

[29] E. M. Osborn, *Brit. J. Exp. Path.*, 1943, **24,** 227.

[30] E. H. Lucas and R. W. Lewis, *Science*, 1944, **100,** 597.

[31] I. E. Muskat, B. C. Becker, and J. S. Loewenstein, *J. Amer. Chem. Soc.*, 1930, **52,** 326.

[32] E. Shaw, *ibid.*, 1946, **68,** 2510.

J. S. Moffatt, G. Newberry, and W. Webster [33] record the following activities for propionylacrylic acid and its ethyl ester :

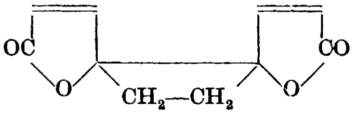
	M.E.D.		
	S. aureus.	*B. coli.*	*Ps. pyocyanea.*
Propionylacrylic acid .	1 in 2000—4000	1 in 2000—4000	1 in 2000
Ethyl propionylacrylate	1 in 32,000—64,000	1 in 32,000—64,000	1 in 8000—16,000

The relatively high activity of the ethyl ester is noteworthy.

Protoanemonin shows antibacterial activity against a wide variety of micro-organisms, including Gram-positive, Gram-negative, and acid-fast bacteria.[34] The following table [26] shows a comparison of the antibacterial activity of natural protoanemonin and material synthesised by two independent methods. The figures represent the highest dilution of protoanemonin which completely inhibited growth in a standard period of time.

	S. aureus.	*E. coli.*		*Candida albicans.*
		(*a*) Casein hydrolysate broth.	(*b*) Infusion broth.	
Natural . . .	60,000	83,000	33,000	100,000
Synthetic. . .	33,000	83,000	33,000	62,000
Synthetic. . .	50,000	83,000	25,000	125,000

Protoanemonin polymerises at room temperature within a few hours, mainly to the dimer, anemonin, for which the structure (X) has been suggested. F. Boas and R. Steude [35] state that anemonin shows little antibacterial activity towards *E. coli*, *C. albicans*, and *S. aureus*, but Baer *et al.*[26] have shown that the growth of *E. coli* is inhibited by anemonin at a concentration of 1 in 2000, *i.e.*, its activity is approximately equal to that of the simple unsaturated lactones.



(X.)

Penicillic Acid.—Penicillic acid was first isolated by C. L. Alsberg and O. F. Black [36] from culture filtrates of *Penicillium puberulum*, after their observation of the inhibiting effect of this mould on the growth of yeast and *B. coli*, and has since been isolated from the metabolic products of *P.*

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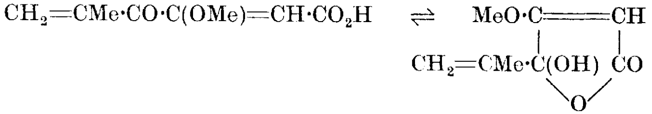
[33] *J.*, 1946, 451.

[34] B. C. Seegal and M. Holden, *Science*, 1945, **101**, 413.

[35] *Biochem Z.*, 1935, **279**, 417.

[36] *U.S. Dep. Agric. Bur. Plant Ind. Bull.*, 1913, No. 270.

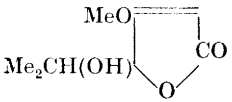
cyclopium,[37] *Aspergillus ochraceus*, *P. thomii*, *P. suavolens*,[38] and *P. aurantio-virens*.[39] The reactions of penicillic acid, $C_8H_{10}O_4$, an optically inactive crystalline solid of m.p. 86°, suggest its formulation [40] as (XI).

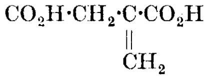

(XI.)

Spectroscopic evidence [32, 41a] shows that penicillic acid in solution exists completely in the lactol form. According to Shaw, treatment with 0·1N-sodium hydroxide causes a marked shift of the absorption maximum towards the visible, corresponding to the formation of the salt of the open-chain form. R. A. Raphael,[41a] however, states that the ultra-violet absorption spectra of penicillic acid in both acid and alkaline solutions are practically identical, and that a repetition of Shaw's determination in 0·1N-sodium hydroxide gave no sign of any shift in the absorption maximum. Shaw has reported that the light-absorption properties of β-acetylacrylic acid (IX), a compound structurally analogous to penicillic acid, are the same in both acid and alkaline solution. The apparent discrepancy in these results is probably caused by the use of heat to effect solution. The complete synthesis of penicillic acid has recently been achieved.[41b]

Whilst penicillic acid has the same toxicity as phenol (0·3 g./kg.) it completely suppresses the growth of *S. aureus* at 1 in 50,000, whereas phenol only partly suppresses growth at 1 in 1000.[42, 43] Penicillic acid is not as powerful against Gram-positive bacteria as penicillin, but it is much more active against Gram-negative. For example, it causes complete inhibition of the growth of *B. coli* at 1 in 50,000, whereas penicillin causes no inhibition at 1 in 1000.[44] Penicillic acid has only weak fungistatic activity.[23]

Oxford *et al.*[42] have considered in some detail the bearing which the powerful general bacteriostatic activity of penicillic acid has on the relationship between structure and antibacterial activity. Since dihydropenicillic acid (XII) and the simple unsaturated lactones have only weak antibacterial activity, it follows that the unsaturated lactone grouping *per se* is not responsible for the marked antibacterial activity. Oxford *et al.* suggest that activity is not solely due to the arrangement of double bonds relative to the carbonyl group, since benzoquinone, which has a similar arrangement,


(XII.)


(XIII.)

$$CO_2H{\cdot}CH_2{\cdot}\underset{\underset{\displaystyle CH_2}{\|}}{C}{\cdot}CO_2H$$

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[37] A. E. Oxford and H. Raistrick, *Biochem. J.*, 1935, **29**, 1549.

[38] E. O. Karow, H. B. Woodruff, and J. W. Foster, *Arch. Biochem.*, 1944, **5**, 279.

[39] Private communication from Professor A. R. Todd and Dr. C. Hassall.

[40] J. H. Birkinshaw, A. E. Oxford, and H. Raistrick, *Biochem J.*, 1936, **30**, 394.

[41] (*a*) R. A. Raphael, *J.*, 1947, 805; (*b*) *idem*, *Nature*, 1947, **160**, 261.

[42] A. E. Oxford, H. Raistrick, and G. Smith, *Chem. and Ind.*, 1942, **61**, 22.

[43] A. E. Oxford, *ibid.*, p. 48.

[44] E. P. Abraham *et al.*, *Lancet*, 1941, **241**, 177.

has a qualitatively different action which is quantitatively much less. Kojic acid, which also has a similar structural arrangement, shows *ca.* one-tenth of the activity of penicillic acid against *S. aureus.* Geiger and Conn [23] have shown that some $\alpha\beta$-unsaturated ketones possess a similar activity to that of penicillic acid, although as pointed out by E. D. Amstutz, E. A. Fehnel, and C. R. Neumoyer [45] the low activity of the carolic acid group shows that high activity must be due to something more than an unsaturated ketone grouping.

The activity is not solely due to the $CH_2{=}C{—}$ grouping, since itaconic acid (XIII) is no more active than kojic acid.

The relative inactivity of dihydropenicillic acid further precludes the possibility that the structural relationship of methoxyl, double bond, and carboxyl causes the marked activity of penicillic acid.

The activity is not due to the $\beta\gamma$-diketo-acid (XIV) produced when the methyl group is hydrolysed to hydroxyl—it being assumed that the low

$$\begin{tikzcd} CH_2{=}CMe{\cdot}CO{\cdot}C(OMe){=}CH{\cdot}CO_2H \arrow[r] & CH_2{=}CMe{\cdot}CO{\cdot}C(OH){=}CH{\cdot}CO_2H \arrow[d, shift left] & \\ & CH_2{=}CMe{\cdot}CO{\cdot}CO{\cdot}CO_2H \arrow[u, shift left] & \text{(XIV.)} \end{tikzcd}$$

activity of dihydropenicillic acid is due to the greater difficulty of hydrolysis of the methoxyl group—since, if the methoxyl group is hydrolysed with sodium hydroxide, the activity against *B. coli* and *S. aureus* is completely destroyed.

However, the work of Geiger and Conn and the activity shown by protoanemonin show that the statement of Oxford *et al.* that " there is no structural feature in this relatively simple molecule which can be omitted or modified without detriment to its remarkable antibacterial properties " must now be reconsidered.

Patulin.[57]—Patulin has been isolated, under the name patulin, claviformin, clavacin, clavatin, or expansin, from *Aspergillus clavatus,*[46] *A. giganteus,*[47] *Penicillium claviforme,*[48] *P. patulum,*[49, 55] *P. expansum,*[50, 51, 55] *P. urtica, A. terreus,*[51] *P. melanii,* and *Gymnoascus* sp.[52]

The structure of patulin is still a matter for some debate. J. H. Birkinshaw *et al.*[53] proposed the structure (XV). Direct reduction with hydriodic acid gave a 60% yield of ε-iodo-γ-keto-n-hexoic acid ; hydrogenation followed by further reduction with hydriodic acid and red phosphorus gave β-methylhexoic acid and β-methyl-γ-hexolactone. The main evidence

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[45] *J. Amer. Chem. Soc.*, 1946, **68,** 349.

[46] B. P. Wiesner, *Nature,* 1942, **149,** 356. S. A. Waksman, E. S. Hornung, and E. L. Spencer, *Science,* 1942, **96,** 202 ; *J. Bact.*, 1943, **45,** 233.

[47] H. W. Florey, M. A. Jennings, and F. J. Philpot, *Nature,* 1944, **153,** 139.

[48] (*a*) W. H. Wilkins and G. C. M. Harris, *Brit. J. Exp. Path.*, 1942, **23,** 166 ; 1943, **24,** 141 ; (*b*) E. Chain, H. W. Florey, and M. A. Jennings, *ibid.*, 1942, **23,** 203.

[49] J. H. Birkinshaw, A. Bracken, M. Greenwood, W. E. Gye, W. A. Hopkins, S. E. Michael, and H. Raistrick, *Lancet,* 1943, ii, 625.

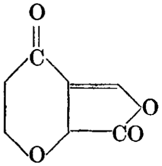
[50] F. Bergel, A. L. Morrison, A. R. Moss, and H. Rinderknecht, *J.*, 1944, 415.

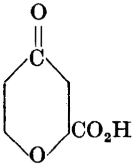
[51] J. Kent and N. G. Heatley, *Nature,* 1945, **156,** 295.

[52] E. O. Karow and J. W. Foster, *Science,* 1944, **99,** 265.

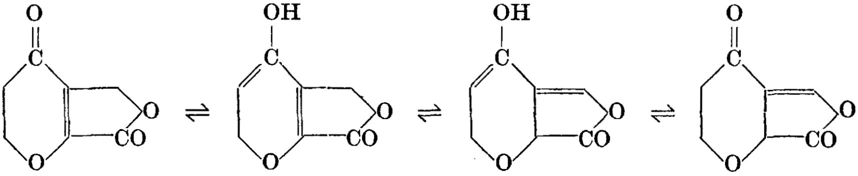
[53] *Lancet,* 1943, ii, 625.

depended on acid hydrolysis, which gave formic acid (and no other volatile acid) and, in 10% yield, tetrahydro-γ-pyrone-2-carboxylic acid (XVI).

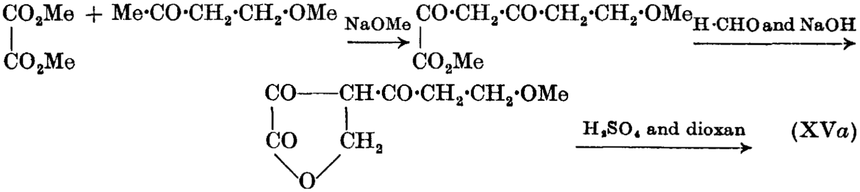

(XV.)


(XVI.)

Confirmatory evidence was provided by conversion of patulin into β-*n*-propylbutyrolactone and γ-keto-β-methyl-*n*-hexoic acid, both of which contain all the original seven carbon atoms ; but to explain the easy acylation and etherification of patulin, F. Bergel *et al.*[50] suggested the formulation as below as the most satisfactory, with (XV*b*) as the predominant tautomer.


(XV*a*.) (XV*b*.) (XV*c*.) (XV.)

Synthesis of (XV*a*) [54] by the following route, however, gives a product which shows only the weak activity of a simple unsaturated lactone, and so it seems that the equilibrium postulated above is incorrect :



Patulin possesses antibacterial properties against both Gram-negative and Gram-positive bacteria, acting in concentrations of 1 in 160,000 to 1 in 40,000.[48] It is toxic to animal tissues ; leucocytes are killed at concentrations of 1 in 800,000, and doses of 0·2 mg. are fatal to mice ;[58] it is considerably more toxic than penicillic acid (lethal dose for mice, 7 mg.).

Patulin also shows marked fungistatic properties ; [55, 56] it suppresses the

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[54] B. Puetzer, C. H. Nield, and R. H. Barry, *Science*, 1945, **101**, 307 ; *J. Amer. Chem. Soc.*, 1945, **67**, 832.

[55] W. K. Anslow, H. Raistrick, and G. Smith, *J. Soc. Chem. Ind*, 1943, **62**, 236.

[56] S. A. Waksman and E. Bugie, *Proc. Soc. Exp. Biol. Med.*, 1943, **54**, 79.

[57] I. R. Hooper, H. W. Anderson, P. Skell, and H. E. Carter, *Science*, 1944, **99**, 16 ; F. Bergel, A. L. Morrison, A. R. Moss, R. Klein, H. Rinderknecht, and J. L. Ward, *Nature*, 1943, **152**, 750 ; E. Chain, H. W. Florey, and M. A. Jennings, *Lancet*, 1944, i, 112 ; W. Th. Nauta, H. K. Oosterhuis, A. C. Linden, P. Van Duyn, and J. W. Dienske, *Rec. Trav. chim.*, 1945, **64**, 254.

[58] A. G. Lochhead, F. E. Chase, and G. B. Landerkin, *Canadian J. Res.*, 1946, **24*E***, 1.

growth of a number of species of *Pythium*, including *P. debaryanum* completely at concentrations of 1 in 400,000 to 1 in 500,000, partly at a concentration of 1 in 1,000,000.

Miscellaneous Unsaturated Lactones showing Antibacterial Activity.—E. M. Osborn [29] has shown that aqueous extracts of many crushed plants possess antibacterial properties. Many of the plants were known to give rise, on crushing, to a bacterial inhibitor such as cyanide, polyphenol, quinone, saponin, *iso*thiocyanate, etc., but no clue could be obtained from the literature as to the probable nature of the inhibitor in others. In two cases it has now been found that the activity is due to the presence of unsaturated lactones, whose structure is not, however, completely known.

The first, and more important, of the two is crepin, $C_{14}H_{16}O_4$, an antibiotic derived from *Crepis taraxacifolia* (Thuill).[59] Crepin does not occur in the plant in the free state, but as an antibiotically-inactive precursor from which it may be derived by enzymic hydrolysis. It is a crystalline solid, m.p. $> 300°$, which contains one *C*-Me group and two hydroxyl groups. On hydrogenation four atoms of hydrogen are absorbed with subsequent loss of antibacterial properties. It has no free carbonyl group, but dissolves slowly in aqueous sodium hydroxide, becoming inactivated, and is reprecipitated unchanged on acidification. It gives no characteristic coloration with ferric chloride, which suggests that the solubility in sodium hydroxide is due to a lactonic, and not a phenolic, group. The presence of an unsaturated lactone ring was confirmed by the Legal nitroprusside test.[60]

The antibacterial activity of crepin is somewhat less than those of penicillic acid and patulin ; Heatley (*loc. cit.*) gives the following figures for the concentrations at which the growth of various organisms is inhibited : *S. aureus*, 1 in 32,000 ; *Str. pyogenes*, 1 in 8000 ; *B. subtilis*, 1 in 16,000 ; *Ps. pyocyanea*, 1 in 4000 ; *S. typhi*, 1 in 4000. It will again be noted that the activity extends to Gram-positive as well as Gram-negative organisms. Crepin is also toxic, though less so than penicillic acid.

The second of these lactones, $C_{10}H_{14}O_4$, discovered through Osborn's work, was isolated by E. P. Abraham, N. G. Heatley, R. Rolt, and E. M. Osborn [61] from *Spiræa aruncus* L.. A crystalline solid, m.p. 79—80°, $[\alpha]_D^{20°} + 55{\cdot}8°$ (in water), it contains one *C*-Me group, and no methoxyl groups, and absorbs six atoms of hydrogen on complete hydrogenation. The compound itself is neutral ; one acidic group appears on treatment with sodium hydroxide, the antibacterial activity being destroyed and ketonic properties appearing. It does not reduce Tollens's reagent, and gives a positive Legal test, suggesting the presence of an unsaturated lactone grouping. The antibacterial activity of this substance is less than that of crepin, preventing the growth of *S. aureus*, *B. proteus*, and *B. coli* only at a concentration of 1 in 4000 and having no effect on *Ps. pyocyanea* at 1 in 2000.

Another unsaturated lactone which is obtained from plant extracts and

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[59] N. G. Heatley, *Brit. J. Exp. Path.*, 1944, **25**, 208.

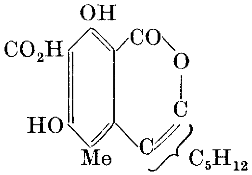
[60] W. A. Jacobs, A. Hoffmann, and E. L. Gustus, *J. Biol. Chem.*, 1926, **70**, 1.

[61] *Nature*, 1946, **57**, 511.

shows antibacterial activity, is the hæmorrhagic agent dicoumarin (XXXVI) isolated from spoiled sweet clover (see below).

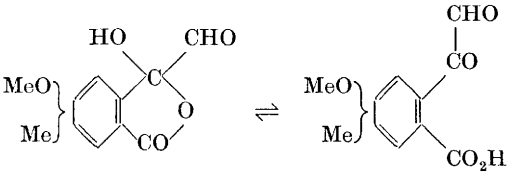
A. Gott [62] has shown that dicoumarin shows an activity considerably greater than that of the simple unsaturated lactones, the growth of *S. aureus, S. pyogenes, S. viridans*, and *B. anthracis* being inhibited by a concentration of 1 in 100,000. *S. fecalis, B. subtilis, C. welchii, E. coli*, and *Ps. pyocyanea* were among organisms unaffected at 1 in 25,000.

An investigation similar to that of Osborn on plant extracts has been carried out by W. H. Wilkins and G. C. M. Harris,[48a] who have investigated the production of antibiotics by fungi. The culture liquid from *Penicillium brevicompactum* Dierckx inhibited the growth of *S. aureus*, and the substance responsible for the inhibition was later isolated by H. W. Florey and his co-workers [63] and identified as mycophenolic acid, a substance isolated from *P. brevicompactum* some years earlier.[36, 64] Mycophenolic acid, $C_{17}H_{20}O_6$, is a crystalline solid, m.p. 141°, which on demethylation gives normycophenolic acid, for which the structure (XVII) has been suggested.[65]



(XVII.)

It was also shown by the work of Wilkins and Harris [48a] that *Penicillium gladioli* produced an antibacterial substance, $C_{11}H_{10}O_5$, during growth. This substance has been isolated [66] as a crystalline solid, m.p. 160°, and given the name gladiolic acid. The complete structure of gladiolic acid is not known; J. C. McGowan [67] suggests the structures (XVIII), but detailed experimental evidence for these has not yet been published. The antibacterial activity is not marked; at pH 7 the growth of *S. aureus* is inhibited at 1 in 4000, but the growth of *Salmonella typhi* or *E. coli* is unaffected at 1 in 2000. Gladiolic acid is markedly fungistatic, the activity depending on pH and hence probably on the proportion of undissociated molecules or on the occurrence of the lactone ring. At pH 3·5, a concentration of 1 in



(XVIII.)

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[62] *Science*, 1945, **101**, 383.

[63] H. W. Florey, K. Gilliver, M. A. Jennings, and A. G. Sanders, unpublished results quoted by E. P. Abraham, *Biochem. J.*, 1945, **39**, 398.

[64] P. W. Clutterbuck, A. E. Oxford, H. Raistrick, and G. Smith, *Biochem. J.*, 1932, **26**, 1441.

[65] P. W. Clutterbuck and H. Raistrick, *ibid.*, 1933, **27**, 654.

[66] P. W. Brian, P. J. Curtis, J. F. Grove, H. G. Hemming, and J. C. McGowan, *Nature*, 1946, **157**, 697.

[67] *Chem. and Ind.*, 1947, 205.

500,000 will prevent germination of *Botrytis allii* conidia, but at pH 7 a concentration of 1 in 10,000 is needed.

Mode of Action.—The possible mode of action of this general type of antibiotic has been the subject of many recent papers.

A. E. Oxford [68] has shown that penicillic acid reacts with ammonia and substances containing amino-groups such as amino-acids, peptone, and *p*-aminobenzoic acid with a considerable diminution of its antibacterial power, and E. Chain and H. W. Florey [69] consider that this reactivity with amino-groups is possibly the cause of the antibacterial and toxic activity, suggesting that both penicillic acid and patulin (which is also inactivated by amino-compounds) act as general protoplasmic poisons. No precise work seems to have been carried out on the nature of this deactivation of amino-compounds.

The antagonism between δ-hexenolactone and the alanines found by Hauschka in his work on animal tissues [19] may be compared with that found between penicillic acid and patulin and amino-compounds.

Geiger and Conn,[23] as already mentioned, consider that, since both penicillic acid and patulin are inactivated by excess of thiol, their antibiotic activities are probably due to reaction with the SH groups of bacterial enzyme systems or with SH-containing essential metabolites. They point out that both compounds contain the grouping $-CH=C-\overset{|}{C}=O$, and that many compounds containing this grouping react readily with thiols.[70] From a survey of the addition reactions of unsaturated ketones, they suggest that addition reactions will take place most readily with a compound of type (XIX) when R′ is an aromatic radical such as phenyl, and R″ and R‴ are hydrogen atoms. In pursuit of this theory, several $\alpha\beta$-unsaturated ketones were tested and found to resemble patulin and penicillic acid closely, both in their reactivity towards thiols and in their bacteriostatic and fungistatic properties. Indeed, phenyl vinyl ketone (XIX ; R′ = Ph, R″ = R‴ = H) was as active as patulin, and more active than penicillic acid, against Gram-positive organisms, although it had only one-third to one-tenth of the activity against Gram-negative organisms. Other unsaturated ketones such as mesityl oxide, phorone, and *iso*phorone had no significant activity, but Geiger and Conn were able to show that only with phenyl vinyl ketone did reaction with thiols proceed to completion as it did with penicillic acid and patulin. C. J. Cavallito and T. H. Haskell [71] point out that whilst the inactivation by thiols of penicillic acid, clavacin, protoanemonin, the antibacterial agent $C_{15}H_{20}O_5$ from *Arctium minus*,[72] and δ-hexenolactone can be explained as involving only addition of R·SH

$$R'\cdot CO\cdot CH{=}CR''R''' \qquad \text{(XIX.)}$$

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[68] *Biochem. J.*, 1942, **36**, 438.

[69] *Ann. Reports*, 1943, **40**, 191.

[70] T. Posner, *Ber.*, 1902, **35**, 799 ; 1904, **37**, 502.

[71] *J. Amer. Chem. Soc.*, 1945, **67**, 1991.

[72] C. J. Cavallito, J. H. Bailey, and F. K. Kirchner, *ibid.*, p. 948.

to a double bond, it cannot be ignored that all these compounds are lactones, and might react as such with thiols. They therefore investigated the reaction of certain lactones with amino-thiols under physiological conditions. Reaction appeared to proceed by addition of the thiol group to the double bond, followed in the case of the $\Delta^{\beta\gamma}$-γ-lactones by reaction of the lactone with the amino-group and loss of water.

These suggestions as to the mode of action of this type of compound almost certainly do not present the whole picture of the mechanism of their antibacterial activity, but they serve as useful working hypotheses—the term being used in its broadest sense—for further work for both chemist and biologist.

(III) *Inhibition of the Germination of Seeds and of Plant Growth*

The suggestion that unsaturated lactones inhibit the germination of seeds—the so-called blastocholine activity—was first made by R. Kuhn.[17, 73] The term " blastocholine " was first used by A. Köckemann [74] to describe factors present in various ripe fruits and seeds which suppressed the germination of other seeds. Blastocholines, which were of unknown structure, were found to be both ether- and water-soluble, insoluble in light petroleum, stable at 100° in aqueous solution, but inactivated by peroxide and alkalis. An especially rich source of one blastocholine was the berry of the mountain ash (*Sorbus Aucuparia*).

Kuhn *et al.*[17] showed that both natural optically active δ-hexenolactone obtained from mountain ash and the synthetic racemic compound showed blastocholine activity, although no claim was made as to the identity of δ-hexenolactone and the blastocholine. In a dilution of 1 in 1000 the germination of cress seeds (*Lepidium Sativum*) was completely suppressed ; at 1 in 10,000 the growth of roots was markedly less than that of the control ; at 1 in 100,000 there was no perceptible effect. Other simple $\alpha\beta$-unsaturated lactones also show blastocholine activity, [17, 18, 75, 76, 77] and from the extent and variety of the examples given in the literature it appears that this activity is a general property of $\alpha\beta$-unsaturated lactones, little dependent on a specific structure of the total molecule although this affects the relative activity of the compound.

One of the most active compounds tested was coumarin ; at a dilution of 1 in 10,000 only 2% of the seeds germinated, whereas parasorbic acid at this dilution permitted 70—80% to germinate.[17] L. J. Audus and J. H. Quastel [78] have made a more detailed study of the activity of coumarin with a view to its possible use as a selective phytocidal agent and shown that it exerts a dual effect—a marked inhibition of both germination and

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[73] *Die Chemie*, 1943, **56**, 236.

[74] *Ber. deutsch. Bot. Ges.*, 1934, **52**, 523. See also P. Larsen, *Planta*, 1939, **30**, 160 ; A. M. Akkerman and H. Veldstra, *Rec. Trav. chim.*, 1947, **66**, 411, and references quoted therein.

[75] H. Veldstra and E. Havinga, *Rec. Trav. chim.*, 1943, **62**, 841.

[76] F. K. Cameron, *J. Physical Chem.*, 1910, **14**, 422.

[77] W. Sigmund, *Biochem. Z.*, 1914, **62**, 339.

[78] *Nature*, 1947, **159**, 320.

root-growth subsequent to germination. Root-growth was slightly more susceptible than germination. In contrast to this, 2 : 4-dichlorophenoxyacetic acid [79] showed no significant effect on germination over the range of concentrations used.

It was also shown that the action of coumarin was a differential one, carrot being about twenty times as sensitive as cress, whereas these two plants appeared to be equally sensitive to 2 : 4-dichlorophenoxyacetic acid. A summary of their results is given by Audus and Quastel in the following Table :

Species.	50% Inhibition of root-growth.		50% Inhibition of germination.	
	Coumarin (p.p.m.).	2 : 4-Dichlorophenoxyacetic (p.p.m.)	Coumarin (p.p.m.).	2 : 4-Dichlorophenoxyacetic acid (p.p.m.).
Cress	11	0·2	11	Greater than 10 in all cases.
Radish	8	0·6	10	
Mustard	8	0·15	> 20	
Carrot	0·5	0·3	5	
Onion	15	0·3	> 20	
Mixed lawn grass	4	> 10	4	
Beetroot	12	0·11	> 20	
Taraxacum officinale	10	6·0	6	
Cabbage	50	0·04	4	

The inhibitive effects of coumarin are reversible, and Audus and Quastel suggest that it must effect its inhibition by forming loose combinations or easily dissociated compounds with enzymes or metabolites in the plant cell.

Protoanemonin was found to be much more effective and ranunculin, the diglucoside of protoanemonin, slightly less effective than coumarin in suppressing the root-growth and the germination of cress.

The blastocholine activity of unsaturated lactones was regarded by Veldstra and Havinga [75] as supporting the supposition expressed by Veldstra,[80] on the strength of theoretical considerations, that the plant-growth inhibitors might structurally be closely related to the plant-growth substances. This possibility had already been considered on other grounds by P. Fröschel.[81] It was noteworthy that the substance of high blastocholine activity, coumarin, was the lactone of *cis-o*-hydroxycinnamic acid, whilst *cis*-cinnamic acid itself was a highly active plant-growth promoter in the pea test.[82] Veldstra and Havinga found that coumarin had an antagonistic effect to the action of the growth-promoter α-naphthaleneacetic acid in the pea test,[75] although such an influence could not be established in the blastocholine test.

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[79] R. E. Slade, W. G. Templeman, and W. A. Sexton, *ibid.*, 1945, **155,** 497 ; P. S. Nutman, H. G. Thornton, and J. H. Quastel, *ibid.*, p. 498 ; W. G. Templeman and W. A. Sexton, *ibid.*, 1945, **156,** 630.

[80] *Enzymologia.* 1944, **11,** 97, 137.

[81] *Natuurwetensch. Tijds.*, 1939, **21,** 93.

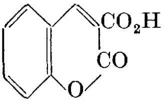
[82] J. B. Koepfli, K. V. Thimann, and F. W. Went, *J. Biol. Chem.*, 1938, **122,** 763.

Veldstra has suggested that there are many indications that, in the protoplasmic membrane, plant-growth substances function as permeability regulators. That is, they increase the permeability of the membrane to water and nutrient material (sugars, etc.) dissolved in it (opening effect). The plant-growth inhibitors are assumed to reverse this effect, decreasing the permeability of the membrane (condensing effect). From experiments with lipophilic systems (used as models for the mainly lipophilic protoplasmic membrane) it appeared that substances which were active as plant-growth promoters showed a marked interaction with a unimolecular lecithin film, whilst a qualitative test with oleate coacervates showed that α-naphthylene-acetic acid had an " opening " effect on this system, whereas coumarin had a " condensing " effect. In mixtures their activities were antagonistic. Veldstra further suggested that it would be expected that inhibitors, like growth promoters, contain a characteristic grouping responsible for their activity.[76] In view of the essential function of the side-chain with a carbonyl group in the growth substances, and the structural relation of *cis*-cinnamic acid to coumarin, it was probable that the essential elements of these inhibitors was the lactone ring.

The purely physical view of the mode of action of the unsaturated lactones functioning as plant-growth inhibitors is of interest. E. Kodicek and A. N. Worden,[83] in a study of the inhibition of the growth of *Lactobacillus helveticus* by linolenic, linoleic, and oleic acids, noted that the inhibition was reversed by the addition of similar concentrations of certain surface active agents such as cholesterol or lecithin. They suggested that if the unsaturated fatty acid were to form a monolayer around the bacterium it might exert its effect in at least three ways : (i) by changing the permeabilities of adjacent surfaces, (ii) by exerting some chemical influence, and (iii) by altering the surface tension and so interfering with bacterial division. The activity is limited to Gram-positive organisms. Whether surface-active agents would affect the activities of the plant-growth inhibitors is not known. It would be of interest to determine whether similar antagonisms to those shown by the antibiotics were shown by the plant-growth inhibitors.

(IV) *Fish Poison and Insecticidal Activity*

Many naturally occurring nitrogen-free fish poisons and insecticides are derivatives of coumarin. Coumarin itself acts as a narcotic for rabbits, frogs, earthworms, and many other animals, and is a sedative and hypnotic for mice. It causes no marked harm to the heart, but diminishes the sensitivity of the sympathetic nerve and causes paralysis of smooth muscle.



(XX.)

In contrast to coumarin, coumarin-3-carboxylic acid (XX), which is a sedative in small doses and acts as a hypnotic in larger amounts, has a sufficiently large therapeutic range of action to permit its use as a sedative

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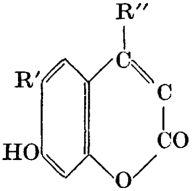
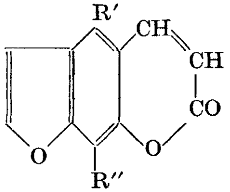
[83] *Biochem. J.*, 1945, **39**, 78.

only. F. W. Werder[85] has prepared and tested more than a hundred derivatives—mainly salts, esters, and substituted amides—of coumarin-3-carboxylic acid. The diethylamide is useful in general nervous diseases and in various neurasthenic and hysterical ailments. It has the same limiting dosage (0·2 g.), as phanodorm (*cyclo*hexenylethylbarbituric acid), but its lethal dose is five times greater. The salt of coumarin-3-carboxylic acid with ephedrine has been used in an antiasthmatic (epocan).

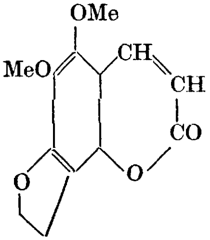
Compound.	Concn.	Compound.	Concn.
Valerolactone	1 in 1,000	Pimpinellin	1 in 50,000
4-Methylumbelliferone	1 in 5,700	*iso*Pimpinellin	1 in 60,000
Coumarin	1 in 6,800	Ostruthin	1 in 67,000
6-Nitrocoumarin	1 in 10,500	Oxypeucedanin	1 in 70,000
5 : 7-Diacetoxycoumarin	1 in 11,000	Xanthotoxin	1 in 70,000
4-Methylcoumarin	1 in 12,500	Imperatorin	1 in 74,000
7-Ethoxycoumarin	1 in 15,700	Osthol	1 in 82,000
3-Chlorocoumarin	1 in 17,000	Bergapten	1 in 93,000
*allo*Imperatorin	1 in 40,000	Methyl*allo*imperatorin	1 in 100,000



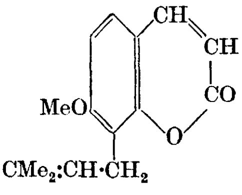


4-Methylumbelliferone ;
$R' = H$; $R'' = Me$.
Ostruthin ;
$R' = CH_2{\cdot}CH{:}CMe{\cdot}CH_2{\cdot}CH_2{\cdot}CH{:}CMe_2$;
$R'' = H$.

Bergapten ;
$R' = OMe$; $R'' = H$.
*allo*Imperatorin ;
$R' = CH_2{\cdot}CH{:}CMe_2$; $R'' = OH$.
Methyl*allo*imperatorin ;
$R' = CH_2{\cdot}CH{:}CMe_2$; $R'' = OMe$.
Xanthotoxin ;
$R' = H$; $R'' = OMe$.
Imperatorin ;
$R' = H$; $R'' = O{\cdot}CH_2{\cdot}CH{:}CMe_2$.
Oxypeucedanin ;
$R' = O{\cdot}CH_2{\cdot}\overbrace{CH{\cdot}O{\cdot}C}Me_2$; $R'' = H$.
*iso*Pimpinellin ;
$R' = R'' = OMe$.



Pimpinellin.



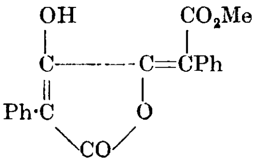
Osthol.

The activity of extracts of plants of the families Rutaceæ and Umbelliferæ as fish poisons has long been known, and was first shown by H. Priess [84] to be due to coumarins. More detailed testing has been done by E. Späth [86] and his co-workers, who have carried out a number of experiments on the effect of coumarins on the fresh-water fish *Lebistes reticulatus* Guppyi. The coumarins act first as stimulants, then as narcotics, and finally as poisons ; it was possible to compare the activities by observing the concentrations required to cause death within a standard time, usually eight hours (see Table, p. 61).

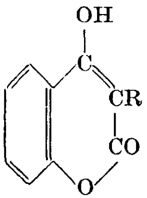
Relative Activities of Coumarins

Läuger, Martin, and Müller [3] in analysis of Späth's results observed that the toxicity increased with increasing substitution, and suggested that the activity could be divided into two parts : (i) the toxic component, (ii) a group which would guarantee lipoid solubility. The toxic component in the coumarins was probably the unsaturated lactone ring. The " absolute " toxicity of this grouping presumably remains constant, but the *effective* toxicity is enhanced in compounds such as *allo*imperatorin methyl ether, in contrast to coumarin or angelicin, because of the accumulation of ether groups and unsaturated longer-chain hydrocarbon residues which greatly increase lipoid solubility. There is an obvious parallel between this suggestion and the Meyer–Overton theory of anæsthesia.

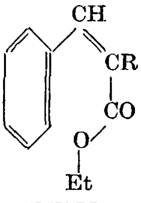
Läuger *et al.* were primarily interested in the discovery of more powerful insecticides—research which culminated in the discovery of the remarkable insecticidal properties of D.D.T. Noting the toxic nature of the unsaturated lactone ring, they set out to prepare insecticides containing this group, using as starting point the naturally occurring insecticide vulpinic acid (XXI). Many compounds were prepared and tested ; coumarin derivatives of the type (XXII) were strongly active, as were several compounds which contained an " opened " lactone ring structure (XXIII). However, none seemed better than the already known insecticides such as rotenone and the pyre-



(XXI.)



(XXII.)



(XXIII.)

(R = CO$_2$Et or COMe)

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[84] *Ber. deutsch. Pharm. Ges.*, 1911, **21**, 267.

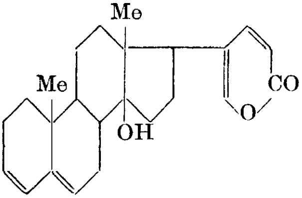
[85] *Merck's Jahresberichte*, 1936, **50**, 88.

[86] See E. Späth, *Ber.*, 1937, **70**, 83.

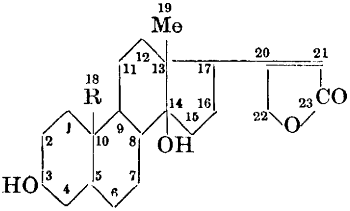
thrins, and their stability in field tests was small. Consequently, with the discovery of the great activity and stability of the D.D.T. type of compound, the work on the coumarin derivatives was abandoned.

(V) *Cardiac Activity* [87]

With the exception of the toad venoms and a few other substances of alkaloidal nature, the cardiac drugs are all non-nitrogenous glycosides which on hydrolysis with acid or enzymes split off the sugar residues to give the genins or aglycones, which are steroid derivatives. The physiological activity of the glycosides resides chiefly in the aglycone residue, although the sugar moiety has a definite significance,[88, 89, 90] the free genins being medicinally valueless. The particular sugars are thought to control water-solubility, cell-penetrability, and persistence of cardiac action. All these drugs produce the same type of effect, manifested on the cardiovascular system. The cardiac muscle is much more sensitive to them than are other muscles of the body, and in cases of congestive heart failure the drug brings relief by increasing the force of systolic contraction and lengthening the time in each cardiac cycle occupied in systole, thereby allowing the heart muscle to rest and the ventricle to fill. One standard method for the pharmacological assay of the heart poisons involves the determination of the minimum subcutaneous dose required to cause systolic standstill after one hour in a frog's heart. Another method is based on the minimum lethal dose for cats. Observation of the effect of the drug on the isolated frog's heart is also used.[87k]



(XXIV.)



(XXV.)

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[87] (*a*) R. C. Elderfield, *Chem. Reviews*, 1935, **17,** 208; (*b*) R. Tschesche, *Ergebn. Physiol.*, 1936, **38,** 31; (*c*) W. H. Strain, in " Organic Chemistry " edited by H. Gilman, New York, 1944, p. 1427; (*d*) L. F. Fieser, " Chemistry of Natural Products related to Phenanthrene ", Reinhold Publishing Corp., New York, 1937, 2nd edition, p. 256; (*e*) W. A. Jacobs, *Physiol. Rev.*, 1933, **13,** 222; (*f*) A. Stoll, " The Cardiac Glycosides ", Pharmaceutical Press, London, 1937; (*g*) L. Goodman and A. Gilman, " The Pharmacological Basis of Therapeutics ", Macmillan Co., New York, 1943, p. 500; (*h*) C. W. Shoppee, *Ann. Rev. Biochem.*, 1942, **11,** 103; (*j*) T. Reichstein and H. Reich, *ibid.*, 1946, **15,** 164; (*k*) L. F. Fieser and M. Fieser, " Organic Chemistry ", D. C. Heath & Co., Boston, 1944, p. 963.

[88] K. K. Chen and R. C. Elderfield, *J. Pharm. Exp. Ther.*, 1942, **76,** 81.

[89] K. K. Chen, E. B. Robbins, and H. Worth, *J. Amer. Pharm. Assoc.*, 1938, **27,** 188.

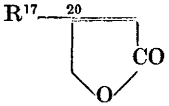
[90] A. C. De Graff, G. H. Paff, and R. A. Lehmann, *J. Pharm. Exp. Ther.*, 1941, **72,** 211.

The aglycones may be divided into two groups on the basis of the structure of their side chains : (i) the " squill-toad venom " group, the smaller of the two, characterised by an α-pyrone side chain carrying the steroid ring in the 5-position, *e.g.*, scillaridin A (XXIV), (ii) the " digitalis-strophanthus " group which has a $\Delta^{\alpha\beta}$-butenolactone side chain substituted on the β-carbon atom (XXV). That the unsaturated γ-lactone ring has a $\Delta^{\alpha\beta}$-structure has been shown by the recent work of R. C. Elderfield [91, 92, 93] and L. Ruzicka [94, 95] and their collaborators.

The following general conclusions have been drawn relating structure and physiological activity. The unsaturated lactone ring is essential : reduction causes a practically complete loss of activity ; [87e, 88] ring opening similarly reduces activity.[88, 96, 97] The tertiary hydroxyl group at C_{14} is considered to be essential,[98] since neriantin,[99] izarin,[100] and diginin,[101] the aglycones of which are thought to lack the C_{14} hydroxyl group, are either inactive or have very low activities. Since adynerin,[99, 102, 103] the aglycone of which differs from other active aglycones by the presence of a double bond at C_8:C_9, is inactive, it is suggested that such unsaturation destroys activity ; convallotoxin, the genin of which is unsaturated at C_9:C_{11}, is strongly active.[104]

Very marked reduction or complete loss of activity may occur when stereochemical rearrangement in the steroid ring has taken place ; [88] *allo*cymarin, the genin of which is *allo*strophanthidin, is inactive.[96] A *cis*-configuration of rings A/B is probably essential ; uzarin, the genin of which probably has a *trans*-configuration of rings A/B, is far less active than those glycosides of which the genins have a *cis*-A/B ring configuration.[97]

Synthetic work in this field may be divided into two groups : (i) attempts to prepare compounds closely related to the cardiac aglycones, (ii) attempts to prepare simple unsaturated lactones showing cardiac activity.



(XXVI.)

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[91] M. Rubin, W. D. Paist, and R. C. Elderfield, *J. Org. Chem.*, 1941, **6,** 260.

[92] R. G. Linfield and R. C. Elderfield, *ibid.*, p. 270.

[93] J. van P. Torrey, J. A. Kuck, and R. C. Elderfield, *ibid.*, p. 289.

[94] L. Ruzicka, T. Reichstein, and A. Fürst, *Helv. Chim. Acta,* 1941, **24,** 76.

[95] L. Ruzicka, P. A. Plattner, and A. Fürst, *ibid.*, p. 716.

[96] W. A. Jacobs, *J. Biol. Chem.*, 1930, **88,** 519.

[97] R. Tschesche and K. Bohle, *Ber.*, 1936, **69,** 2443.

[98] Compare reference 87 (*j*), footnote 16.

[99] R. Tschesche, K. Bohle, and W. Neumann, *Ber.*, 1938, **71,** 1927.

[100] K. K. Chen, A. L. Chen, and R. C. Anderson, *J. Amer. Pharm. Assoc.*, 1936, **25,** 579.

[101] K. K. Chen, quoted in reference 87 (*k*).

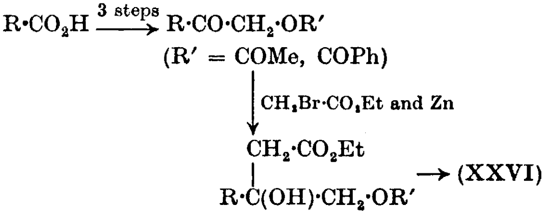
[102] W. Neumann, *Ber.*, 1937, **70,** 1547.

[103] R. Tschesche and K. Bohle, *ibid.*, 1938, **71,** 654.

[104] K. K. Chen, C. I. Bliss, and E. B. Robbins, *J. Pharm. Exp. Ther.*, 1940, **69,** 279.

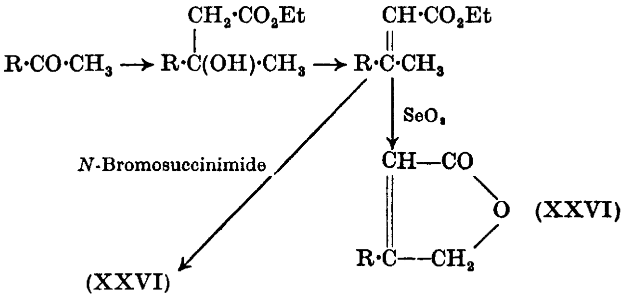
Numerous compounds of the type (XXVI; R = steroid) have been prepared by the following methods:

(I) [105, 94, 95]



$$R\cdot CO_2H \xrightarrow{\text{3 steps}} R\cdot CO\cdot CH_2\cdot OR' \quad (R' = COMe,\ COPh) \xrightarrow{CH_2Br\cdot CO_2Et \text{ and Zn}} R\cdot C(OH)(CH_2\cdot CO_2Et)\cdot CH_2\cdot OR' \longrightarrow (XXVI)$$

(II) [106]

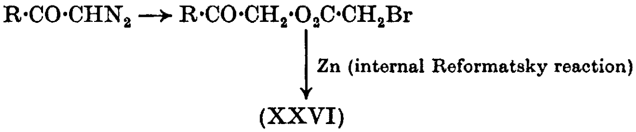


$$R\cdot CO\cdot CH_3 \longrightarrow R\cdot C(OH)(CH_2\cdot CO_2Et)\cdot CH_3 \longrightarrow R\cdot C(CH_3){=}CH\cdot CO_2Et \xrightarrow{SeO_2} (XXVI)$$

$$R\cdot C(CH_3){=}CH\cdot CO_2Et \xrightarrow{N\text{-Bromosuccinimide}} (XXVI)$$

(III) [107]

(IV) [108]



$$R\cdot CO\cdot CHN_2 \longrightarrow R\cdot CO\cdot CH_2\cdot O_2C\cdot CH_2Br \xrightarrow{\text{Zn (internal Reformatsky reaction)}} (XXVI)$$

All these lactones prepared gave the Legal test and showed an ultra-violet absorption maximum at *ca.* 2150—2200A. Since they all[*] lack the C_{14} hydroxyl group they would not be expected, from the evidence at present available, to show cardiac activity.

Experiments in the second group have not been successful; although many simply substituted unsaturated lactones have been prepared and tested,[109, 110] none so far prepared shows the characteristic digitalis-like activity on the mammalian heart.

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[105] J. Fried, R. G. Linville, and R. C. Elderfield, *J. Org. Chem.*, 1942, **7**, 362.

[106] L. Ruzicka, P. A. Plattner, and J. Pataki, *Helv. Chim. Acta*, 1942, **25**, 425; 1944, **27**, 988.

[107] *Idem, ibid.*, 1945, **28**, 1360.

[108] P. A. Plattner and H. Heusser, *ibid.*, p. 1044.

[109] (*a*) R. C. Elderfield *et al.*, *J. Org., Chem.*, 1941, **6**, 261, 270, 273, 289; 1942, **7**, 362, 372, 383, 444; (*b*) S. K. Ranganathan, *Current Sci.*, 1940, **9**, 458; (*c*) G. Swain, A. R. Todd, and W. S. Waring, *J.*, 1944, 548.

[110] (*a*) K. K. Chen, F. A. Steldt, J. Fried, and R. C. Elderfield, *J. Pharm. Exp. Ther.*, 1942, **74**, 381; (*b*) O. Krayer, R. Mendez, E. M. de Espanes, and R. P. Linstead, *ibid.*, p. 374; (*c*) O. Krayer, R. Mendez, and D. Todd, *ibid.*, 1943, **77**, 113.

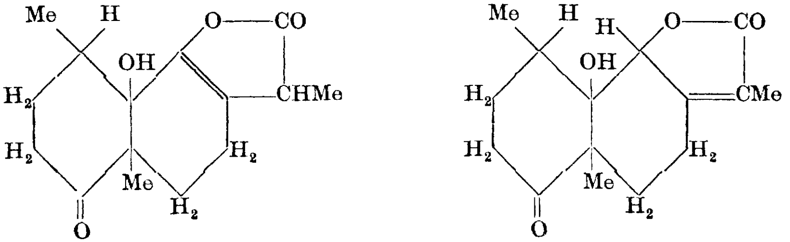
[*] P. A. Plattner, L. Ruzicka, H. Heusser, and E. Angliker (*Helv. Chim. Acta*, 1947, **30**, 1073) have recently described the synthesis, by method (I), of *allo*uzarigenin. This represents the first synthesis of a compound containing the C_{14} hydroxyl group and the lactone ring of the natural aglycones.

Certain of these simple lactones—*e.g.*, α- and β-angelicalactones, methyl and ethyl coumalates, and L-ascorbic acid—cause systolic standstill of the frog's ventricles when adequate doses are injected into the ventrical lymph sac,[110, 111] but the activities of ascorbic acid [110b, 112] and the angelicalactones [113] can be accounted for by the formation of peroxides in the solutions. Hydrogen peroxide itself,[110b] the peroxide formed during the autoxidation of diethyl ether,[114] benzoyl hydrogen peroxide,[115] and *tert.*-butyl hydroperoxide [113] all cause systolic standstill of the isolated frog's heart. When formation of peroxides is prevented by the addition of thiol-containing molecules such as cysteine, glutathione, and diethylthiocarbamate, the effect of ascorbic acid and the angelicalactones is greatly decreased.

Pursuing this method of approach, Mendez later showed [116] that systolic standstill in the frog's heart is also caused by the action of compounds such as *o*-iodosobenzoic acid and iodoacetamide which are known to react readily with sulphhydryl groups.[117]

Recent work by G. R. Clemo and W. Cocker [118] has indicated that simpler compounds possessing the characteristic digitalis-like activity do exist. In the course of an examination of a number of species of *Artemisia*, Messrs T. & H. Smith Ltd. discovered another crystalline compound of the santonin type. The new compound, $C_{15}H_{20}O_4$, termed ψ-santonin, gave cardiac effects, depression of conduction and cardiac inhibition similar to those obtained with digitalis. Neither (—)- nor β-santonin showed similar activity.

Clemo and Cocker showed that the structures of ψ-santonin and (—)-santonin are related; ψ-santonin possesses a tertiary hydroxyl group and an unsaturated γ-lactone ring, although whether this is αβ- or βγ-unsaturated is not yet certain. (XXVII *a* and *b*) are suggested as possible structures. It seems not unreasonable to compare the tertiary hydroxyl group at



(*a*) (*b*)

(XXVII.)

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[111] R. P. Linstead and O. Krayer, *Science*, 1942, **95**, 332.

[112] O. Krayer, *Proc. Soc. Exp. Biol. Med.*, 1943, **53**, 51.

[113] R. Mendez, *J. Pharm. Exp. Ther.*, 1944, **81**, 151.

[114] J. Mita, *Arch. Exp. Path. Pharm.*, 1924, **104**, 276.

[115] H. Richter, *ibid.*, 1940, **194**, 362.

[116] *Science*, 1946, **104**, 5.

[117] E. S. G. Barron and T. P. Singer, *J. Biol. Chem.*, 1945, **157**, 221, 241.

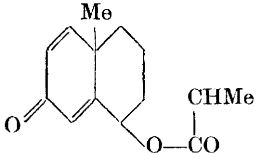
[118] *J.*, 1946, 30.

C_{10} in ψ-santonin with that at C_{14} in the cardiac aglycones, and to ascribe the cardiac activity of ψ-santonin to a possible reproduction of some specific spatial relationship found in the cardiac aglycones.

(VI) *Miscellaneous*

(i) *Anthelmintics.*—Anthelmintics are used to rid the body of a specific group of parasitic worms known as helminths. Helminthiasis is the most common disease in the world; approximately 800 million people are hosts to various types of worms.[119]

Many widely differing types of compound—*e.g.*, thymol, carbon tetrachloride, pelletierine, and phenothiazine—show anthelmintic activity. One of the oldest anthelmintic drugs is the lactone santonin (XXVIII),[120] a crystalline solid obtained from various species of *Artemisia*. The clinical usefulness of santonin is limited because of its toxicity and because it is only effective against ascaris (round worm) and to a lesser degree oxyuris (thread worm), and does not affect tænia (tape-worm); it is also very expensive. Efforts have therefore been made to find more active, less toxic anthelmintics, and many lactones, both saturated and unsaturated, have been prepared and tested for this purpose,[122] following the suggestion by P. Trendelenburg [121] that the lactone group in santonin and similarly constituted compounds has an important bearing on the anthelmintic activity. Much of the preliminary testing has been carried out on earthworms, T. Sollmann [123] having shown that all anthelmintics are very toxic to earthworms.



(XXVIII.)

W. F. von Oettingen [124] tested several lactones and found them all to have a depressant and vermicidal effect on earthworms, due chiefly to muscular depression. The introduction of a double bond into the lactone ring increased the efficiency considerably. In later tests it was found

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[119] Reference 87 (*g*), p. 878.

[120] G. R. Clemo, R. D. Haworth, and E. Walton, *J.*, 1929, 2368; 1930, 1110; G. R. Clemo and R. D. Haworth, *J.*, 1930, 2579; L. Ruzicka and E. Eichenberger, *Helv. Chim. Acta*, 1930, **13**, 1117.

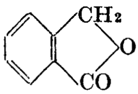
[121] *Arch. Exp. Path. Pharm.*, 1915, **79**, 190.

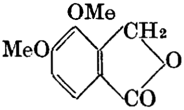
[122] H. Bergs, *Ber.*, 1930, **63**, 1285; K. S. Nargund, N. L. Phalnikar, and their co-workers, *J. Univ. Bombay*, 1940, **9**, 145; 1941, **10**, 99; 1942, **11**, 124, 127; 1943, **11***A*, 104; 1943, **12***A*, 60, 61; 1944, **12***A*, 33; 1944, **13**, 22; K. D. Paranjpe, N. L. Phalnikar, B. V. Bhide, and K. S. Nargund, *J. Indian Med. Assoc.*, 1944, **14**, 69; *Proc. Indian Acad. Sci.*, 1944, **19***A*, 381.

[123] *J. Pharm. Exp. Ther.*, 1918, **12**, 129.

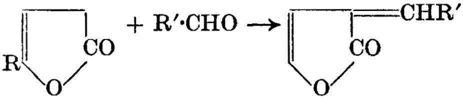
[124] *Ibid.*, 1929, **36**, 335.

that $\Delta^{\alpha\beta}$-angelicalactone was too toxic, and $\Delta^{\beta\gamma}$-angelicalactone too unstable, to be of value. The general activity of various lactones on the musculature of worms has also been examined by L. Lautenschlaeger[125] who showed that, compared with santonin, the phthalide (XXIX) and

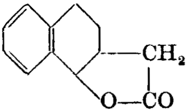

(XXIX.)

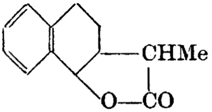

(XXX.)

meconine (XXX) possessed similar parasitotropic activity, but were markedly less organotropic. Lautenschlaeger further stated that the lactone group must be associated with a carbon ring system, open-chain lactones having no anthelmintic action (a result apparently at variance with those of Oettingen). The stability and effectiveness of $\Delta^{\beta\gamma}$-angelicalactone was found to be increased by condensation at the active methylene group with various aromatic hydroxy- and methoxy-aldehydes.[126]



A similar extension of this field was carried out by K. W. Rosenmund and D. Shapiro[127] who tested various aromatic γ-substituted γ-butyrolactones, and noted some surprising variations in activity; for example, *o*-tolylbutyrolactone was more active than santonin, the *p*-compound somewhat less active, and the *m*-compound practically inactive. A. Gluschke[128] prepared a number of santonin-like compounds and found that (XXXI) and (XXXII) were as effective as santonin and practically non-toxic. Rosenmund[129] has suggested that the relative activity of a series of aromatic γ-substituted γ-butyrolactones was not related to surface activity, action on membrane permeability, or action on the swelling of proteins, but to the polarity of the molecules as varied by the relative positions of substituents in the aromatic nucleus.


(XXXI.)


(XXXII.)

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[125] *Ber. deutsch. Pharm. Ges.*, 1921, **31**, 279.

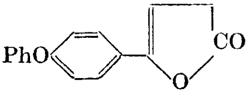
[126] W. F. von Oettingen, *J. Pharm. Exp. Ther.*, 1930, **39**, 59; *J. Amer. Chem. Soc.*, 1930, **52**,, 2024.

[127] *Arch. Pharm.*, 1934, **272**, 313.

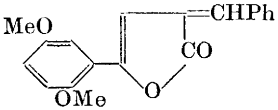
[128] *Arch. wiss. prakt. Tierheilkunde*, 1932, **65**, 201.

[129] *Angew. Chem.*, 1935, **48**, 701.

Following Oettingen's work, M. M. Shah and N. L. Phalhikar [130] have prepared several substituted γ-phenylbut-β-enolactones and their arylidene derivatives, of which only (XXXIII) and (XXXIV) were active ; Rosenmund and Shapiro have shown that the similarly substituted saturated lactones show similar activities, in contrast to Oettingen's statements that the presence of a double bond increases the anthelmintic property.

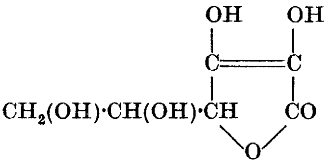


(XXXIII.)



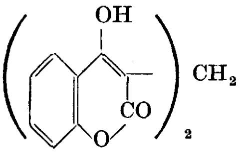
(XXXIV.)

(ii) *Ascorbic Acid.*—The properties and physiological activities of ascorbic acid (XXXV) and its analogues have been reviewed in detail [131] and will not be considered here.



(XXXV.)

(iii) *Hæmorrhagic Agents.*—The hæmorrhagic agent causing " sweet-clover disease " in animals was identified as a coumarin, 3 : 3′-methylenebis-(4-hydroxycoumarin) (XXXVI), in 1941 by M. A. Stahmann, C. F. Huebner, and K. P. Link.[132] Since then (XXXVI), or dicoumarin as it is commonly named, has been the subject of detailed pharmacological testing, and has now been included in the British Pharmaceutical Codex [133] as being useful in the treatment of thrombosis and allied diseases.



(XXXVI.)

Dicoumarin exerts its hæmorrhagic activity by decreasing the prothrombin content of the blood [134] probably by inhibition of the formation of prothrombin in the liver.[135] The hæmorrhagic activity of dicoumarin only makes itself apparent some hours after administration, in contrast to that

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[130] *J. Univ. Bombay*, 1944, **13** (Pt. 3), 22.

[131] T. Reichstein and V. Demole, " Festschrift für E. C. Barell ", Basel, 1936, p. 107 ; E. L. Hirst, " Fortschritte der Chemie organischer Naturstoffe ", Vienna, 1939, p. 132 ; F. Smith, " Advances in Carbohydrate Chemistry ", Vol. II, Academic Press, New York, 1946, p. 79.

[132] *J. Biol. Chem.*, 1941, **138**, 513.

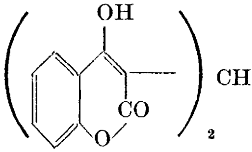
[133] Seventh supplement, 1945, p. 18.

[134] J. Lehmann, *Lancet*, 1942, i, 318.

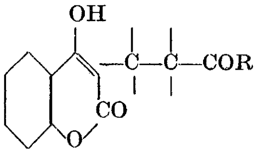
[135] *Acta Physiol. Scand.*, 1944, **8**, 173.

of heparin which appears almost immediately. Whether dicoumarin and vitamin K, or compounds with vitamin K-like activity, are antagonistic has been a matter of some dispute, but it now seems fairly well established that they are. S. Shapiro, M. H. Redish, and H. A. Campbell [136] state that, in man, relatively large doses of vitamin K will counteract the smallest effective doses of dicoumarin.[137] J. Glavind and K. F. Jansen [135] show in experiments on vitamin-K-deficient chicks that there is a quantitative relationship between the two antagonistic substances, so that in certain proportions the rate of prothrombin formation is unaffected. With the smallest active doses the vitamin K : dicoumarin ratio is 1 : 200—500. Glavind and Jansen point out that the lack of constancy in the relationship precludes the possibility of a simple stoicheiometric relation.

Of more than 100 compounds structurally related to dicoumarin tested by Overman and his co-workers,[138] dicoumarin was the most active. It was shown that compounds of high potency can be divided into two classes, (XXXVII) and (XXXVIII). The minimum structural requirements



(XXXVII.)

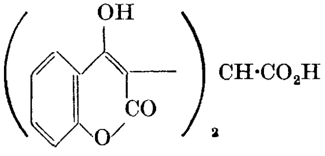


(XXXVIII.)

(R = alkyl)

for a compound to be active were (i) an intact 4-hydroxycoumaryl residue, (ii) the 3-position substituted by C or H. For high activity, a bis-4-hydroxycoumarin structure was needed, or a related structure such as (XXXVIII) with the 4-hydroxyl group and the carbonyl group in the 1 : 5-positions.

Lehmann,[139] in attempting to prepare water-soluble derivatives of dicoumarin, found that



was almost inactive.

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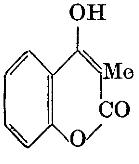
[136] *Proc. Soc. Exp. Biol. Med.*, 1943, **52**, 12.

[137] Compare C. S. Davidson and H. Macdonald, *Amer. J. Med. Sci.*, 1943, **205**, 24 ; P. Meunier and C. Mentzer, *Bull. Soc. Chim. biol.*, 1942, **24**, 371 ; L. B. Jaques and A. P. Dunlop, *Canadian J. Res.*, 1945, **23*E***, 167 ; D. C. Brodie, W. A. Hiestland, and G. L. Jenkins, *J. Amer. Pharm. Assoc.*, 1945, **34**, 73 ; R. S. Overman, J. B. Field, C. A. Baumann, and K. P. Link, *J. Nutrition*, 1942, **23**, 589.

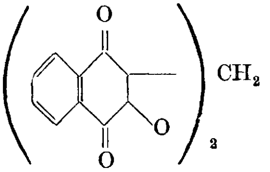
[138] R. S. Overman, M. A. Stahmann, C. F. Huebner, W. R. Sullivan, L. Spero, D. G. Doherty, M. Ikawa, L. F. Graf, S. Roseman, and K. P. Link, *J. Biol. Chem.*, 1944, **153**, 5 ; see also A. Grüssner, Jubilee Vol. Emil Barell, 1946, 238.

[139] *Acta Physiol. Scand.*, 1943, **6**, 28 ; *Lancet*, 1943, i, 458.

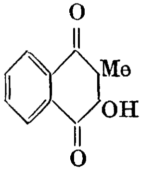
P. Meunier and his co-workers [140] found that (XXXIX) showed vitamin K-like activity (cf. the results of Overman *et al.*) and that (XL), related to the vitamin K-active phthiocol (XLI) as (XXXIX) is to dicoumarin,



(XXXIX.)



(XL.)



(XLI.)

showed slight hæmorrhagic activity. Similar feebly active naphthaquinone derivatives have been prepared by R. Kuhn *et al.*[141]

Symmetrical bismethylene monocyclic lactones have been shown to be inactive,[142] as have various coumarins not hydroxylated at C_4.[143]

The mode of action of dicoumarin remains obscure. Overman *et al.*[138] point out that salicylic acid shows similar effects to dicoumarin, and that all the compounds tested by them which showed anticoagulant activity contain a salicyl configuration, and yield salicylic acid on oxidation *in vitro.* They therefore suggest that the 4-hydroxycoumarin anticoagulant may be metabolised in the body to salicylic acid, which is responsible for the physiological effect. P. Fantl [144] has noted that both formaldehyde and acetaldehyde increase the coagulation time of plasma, and suggested that these, rather than salicylates, might be responsible for the biological effects of dicoumarin. From this, however, it would be feasible to suppose that easily decomposed aldehyde derivatives such as hexamethylenetetramine or paraldehyde would also show the hæmorrhagic effects of dicoumarin, and this has not been demonstrated.

R. S. Overman, M. A. Stahman, and K. P. Link [145] have shown that L-ascorbic acid as well as the antihæmorrhagic 2-methyl-1 : 4-napthaquinone exert at high levels an antagonistic effect to dicoumarin. They suggest that similarities in the pathological effects of deficiencies of L-ascorbic acid and 2-methyl-1 : 4-naphthaquinone, and of continuous administration of dicoumarin, might indicate that the action of dicoumarin may be partly due to disturbances in the metabolism of L-ascorbic acid and the quinone in the animal body. There are not yet enough data to enable conclusions to be drawn as to the relative merits of these theories.

Two other naturally occurring coumarins which show hæmorrhagic activity are ayapanin (7-methoxycoumarin) and ayapin (6 : 7-methylenedioxycoumarin). These were identified by P. K. Bose and P. B. Sen [146]

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[140] P. Meunier, C. Mentzer, N. G. Buu-Hoï, and P. Cagniant, *Bull. Soc. Chim. biol.*, 1943, **25**, 384 ; P. Meunier and C. Mentzer, *ibid.*, p. 80 ; P. Meunier, C. Mentzer, and A. Vinet, *Helv. Chim. Acta*, 1946, **29**, 1291.

[141] *Ber.*, 1943, **76**, 1044.

[142] K. F. Jansen and K. A. Jensen, *Z. physiol. Chem.*, 1942, **66**, 277.

[143] N. von Kaulla, *Klin. Woch.*, 1943, **22**, 205.

[144] *Australian J. Exp. Biol.*, 1944, **22**, 125.

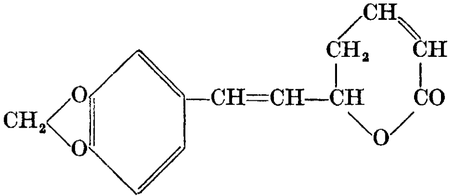
[145] *J. Biol. Chem.*, 1942, **145**, 155.

[146] *Ann. Biochem. Exp. Med.*, 1941, **1**, No. 4, 311.

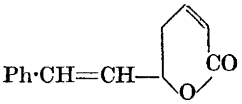
as the active principles of *Eupatorium ayapana*, an aromatic herb whose leaves have been used as a hæmostatic agent. Ayapanin showed its maximum effect some 30—90 minutes after administration, in contrast to the delayed action of dicoumarin. The optimum doses were 0·3 mg./kg. when injected, and 0·5 mg./kg. when taken orally. Later [147] it was shown that umbelliferone (7-hydroxycoumarin) was inactive, and that alkyl groups larger than methyl decreased the activity of ayapanin. Similarly, of several 7-alkoxy-4-methylcoumarins, the methoxy-compound was very active whereas the others showed little activity. Other compounds which showed this activity were non-coumarins, for example, 2-hydroxy-4-methoxybenzaldehyde, 2-hydroxy-4-methoxyacetophenone, 4-methoxycoumaric acid, and 3-methoxyphenyl cinnamate; whilst detailed clinical data are not available, it seems that the activity of this group, discovered by Sen and Bose, is distinct from that due to dicoumarin.

(iv) *Kawa-kawa.*—An extract of peeled root of *Piper methysticum* is used medicinally as an antiseptic and diuretic in inflammatory conditions of the genito-urinary tract.

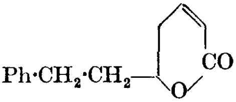
W. Borsche and his co-workers [148] and A. G. Van Veen [149] have shown that among other products the following unsaturated lactones can be isolated from the root:



Methysticin



Kawain



Marindinin (dihydrokawain)

Kawain has been used in the treatment of gonorrhoea under the name "gonosan", but detailed antibiotic tests do not appear to have been made; it is not certain whether the activity of the medicinal preparation from Kawa root is due to the presence of these lactones or not.

The reviewer thanks Professor E. R. H. Jones for many friendly and helpful discussions, and the Department of Scientific and Industrial Research for a Senior Award.

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[147] P. K. Bose, P. B. Sen, and K. Chakravarti, *Ann. Biochem. Exp. Med.*, 1945, **5**, 1.

[148] W. Borsche and A. Roth, *Ber.*, 1921, **54**, 2229; W. Borsche, C. H. Meyer, and W. Peitzsch, *ibid.*, 1927, **60**, 2113; W. Borsche and W. Peitzsch, *ibid.*, 1929, **62**, 360; 1930, **63**, 2414; W. Borsche and B. K. Blount, *ibid.*, p. 2418.

[149] *Rec. Trav. chim.*, 1939, **58**, 521.