Tetrahedron, Supplement No. 7, pp. 333 to 348. Pergamon Press Ltd. Printed in Northern Ireland

THE EXTRACTIVES OF *PISCIDIA ERYTHRINA* L.—III

THE CONSTITUTIONS OF LISETIN, PISCIDONE AND PISCERYTHRONE

C. P. FALSHAW[a], W. D. OLLIS[a], J. A. MOORE[b] and K. MAGNUS[c]

University of Sheffield, Sheffield, England,[a]
University of Delaware, Newark, Delaware, U.S.A.,[b]
and University of the West Indies, Jamaica, West Indies[c]

(*Received* 25 *January* 1966)

Abstract—Further examination of the extractives of the root of Jamaican Dogwood, *Piscidia erythrina* L., has yielded jamaicin (I), ichthynone (II), rotenone (III), sumatrol (IV), three new isoflavonoids, lisetin (XVa), piscerythrone (XXIIIa) and piscidone (XXVIa), and three new rotenoids.

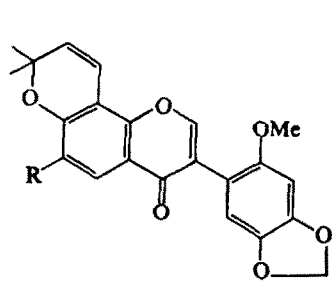
The constitution of lisetin as the coumarono-chromone (XVa) represents a new structural type among the isoflavonoids. The biosynthesis of lisetin may well involve an oxidative coupling reaction since oxidation of piscerythrone (XXIIIa) with alkaline potassium ferricyanide yields lisetin (XVa).

Millettone (XXVIIIa) and isomillettone (XXVIIIb) are new rotenoids and their co-occurrence with the dehydrorotenoid, dehydromillettone (XXIX), is unusual.

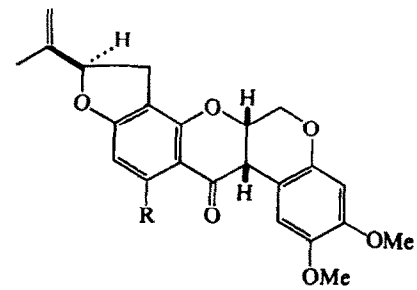
IN a report of an earlier study[1,2] of the root bark of Jamaican Dogwood, *Piscidia erythrina* L., it was remarked that although this plant had been examined by a large number of investigators, there was considerable variation in the natural products isolated. In the more recent investigations, Moore and Eng[3] isolated piscidic acid, jamaicin, lisetin and rotenone; Kapoor, Aebi and Büchi[4] isolated rotenone and jamaicin;[5] and in Part I of this series[2] the isolation of ichthynone and rotenone was described. In an attempt to investigate whether the apparent lack of consistency among the natural products isolated from this plant source was real, a further examination of a sample of the original plant material studied by Moore and Eng[3] was made.[6] This material yielded jamaicin, rotenone and lisetin as described previously,[3] and a new natural product called piscidone, but presumably due to the age of this plant material (it had been stored since 1952), the yield of these substances was low and their isolation was not easy. However, we were fortunate when a freshly collected sample[7] of *P. erythrina* root material became available and its study was particularly rewarding. All the oxygen heterocyclics previously isolated on various occasions including jamaicin (I), ichthynone (II), rotenone (III) and lisetin were again isolated and in addition a number of new natural products was obtained from this source. These included two new isoflavones, piscerythrone and piscidone, the known rotenoid, sumatrol[8] (IV), three new rotenoids, millettone, isomillettone and dehydromillettone,

[1] Part II, S. F. Dyke, W. D. Ollis, M. Sainsbury and J. S. Paul Schwarz, *Tetrahedron* **20**, 1331 (1964).
[2] J. S. Paul Schwarz, Allen I. Cohen, W. D. Ollis, E. A. Kaczka and L. M. Jackman, *Tetrahedron* **20**, 1317 (1964).
[3] J. A. Moore and S. Eng, *J. Amer. Chem. Soc.* **78**, 395 (1956).
[4] A. L. Kapoor, A. Aebi and J. Büchi, *Helv. Chim. Acta* **40**, 1574 (1957).
[5] O. A. Stamm, H. Schmid and J. Büchi, *Helv. Chim. Acta* **41**, 2006 (1958).
[6] This material was kindly made available to us by Dr. L. M. Long, Parke Davis and Company, Detroit, Michigan.
[7] Total root collected by one of us (K. M.) in Jamaica.
[8] [a] T. S. Kenny, A. Robertson and S. W. George, *J. Chem. Soc.* 1601 (1939); [b] L. Crombie and R. Pearce, *Ibid.* 5445 (1961); [c] C. Djerassi, W. D. Ollis and R. C. Russell, *Ibid.* 1448 (1961).

and several other compounds whose structures have not yet been fully established. The constitution of millettone was established in another study[9] and from this the structures of isomillettone and dehydromillettone could be deduced. We now wish



Jamaicin I R = H
Ichthynone II R = OMe



Rotenone III R = H
Sumatrol IV R = OH

to discuss the elucidation of the structures of lisetin, piscerythrone and piscidone.[10]

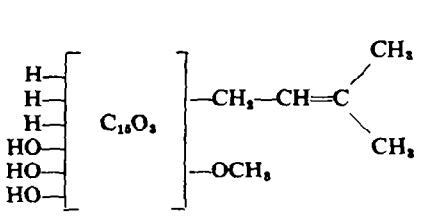
The constitution of lisetin

Mild acetylation of lisetin gave an acetylated product whose NMR spectrum (Table 1) showed that it was a triacetate with a coincident signal ([6], τ 7·67)* due to two acetoxyl methyl groups and singlets due to a third acetoxyl methyl group ([3], τ 7·52) and a methoxyl group ([3], τ 6·10). This showed that lisetin formed a triacetate, $C_{20}H_{12}O_3(OAc)_3(OMe)$, which was also in accord with the formation of lisetin trimethyl ether. Thus lisetin could be represented by the formula $C_{20}H_{12}O_3(OH)_3(OMe)$, that is $C_{21}H_{18}O_7$, rather than by the formula $C_{25}H_{24}O_8$ tentatively proposed earlier.[3]

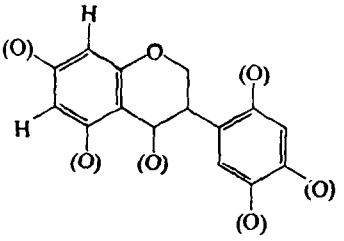
The NMR spectrum of lisetin triacetate also showed signals characteristic of a $\gamma\gamma$-dimethylallyl group[11] with singlets due to two non-equivalent olefinic methyl groups ([3], τ 8·30; [3], τ 8·19), and a triplet ([1], τ 4·78, J = 7 c/s) due to a vinylic proton split by an adjacent methylene group associated with a doublet ([2], τ 6·51, J = 7 c/s). The chemical shift of the methylene protons showed that it was simultaneously allylic and benzylic requiring that the $\gamma\gamma$-dimethylallyl group was an aromatic substituent. The other features of the NMR spectrum of lisetin triacetate included a low field singlet ([1], τ 2·48) and an AB system ([1 and 1], τ 2·64 and 3·08, J = 2·5 c/s) which was assigned to a pair of *meta*-related aromatic protons. The spectrum of lisetin trimethyl ether (Table 1) showed the expected correspondence with that of lisetin triacetate and this permitted the proposal of the partial structure (V) for lisetin.

* Throughout this paper in this context the figures which are enclosed in square brackets indicate proton integrals.

[9] C. A. Rhodes and W. D. Ollis, forthcoming publication.
[10] C. P. Falshaw and W. D. Ollis, *Chem. Comm.*, 305 (1966).
[11] B. F. Burrows, W. D. Ollis and L. M. Jackman, *Proc. Chem. Soc.* 177 (1960); R. B. Bates, R. H. Carnighan, R. O. Rakutis and J. H. Schauble, *Chem. & Ind.* 1020 (1962); R. B. Bates and D. M. Gale, *J. Amer. Chem. Soc.* **82**, 5749 (1960); W. D. Ollis, M. V. J. Ramsay, I. O. Sutherland and S. Mongkolsuk, *Tetrahedron* **21**, 1453 (1965).

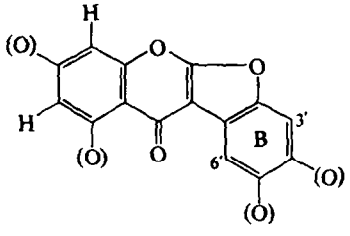


V

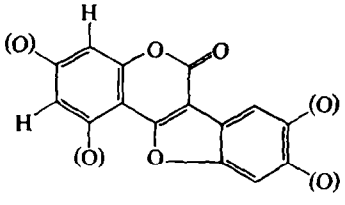


VI

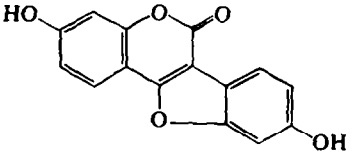
Concerning the undefined [$C_{15}O_3$] portion of this partial structure (V) it was clear that one of the oxygen atoms was present in a carbonyl group since lisetin (ν_{CO} = 1653 cm^{-1}), lisetin trimethyl ether (ν_{CO} = 1660 cm^{-1}) and lisetin triacetate (ν_{CO} = 1665 cm^{-1}) all showed carbonyl bands in the range 1650–1665 cm^{-1}. The co-occurrence of lisetin with the isoflavones, jamaicin (I) and ichthynone (II), suggested that lisetin could be isoflavonoid in type although on empirical grounds it could not be an isoflavone. The *meta* relationship of two of its aromatic protons and their chemical shifts indicated the association with a phloroglucinol residue.[12] On these two grounds an oxygenation pattern such as that shown in formula VI was an attractive proposal for lisetin. This partial structure VI could be developed in two ways in order to account for the presence of a carbonyl group in lisetin so giving the partial structures VII or VIII for consideration.



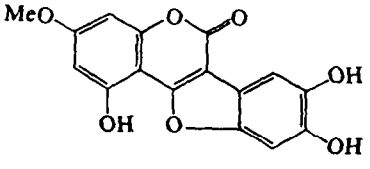
VII



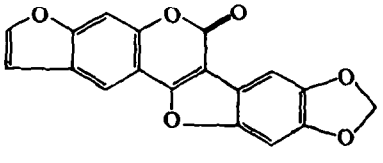
VIII



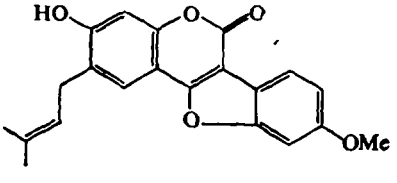
Coumestrol IX



Wedelolactone X



Erosnin XI



Psoralidin XII

[12] J. Massicot and J-P. Marthe, *Bull. Soc. Chim. Fr.* 1962 (1962); H. Erdtman and T. Norin, *Acta Chem. Scand.* 17, 1781 (1963); A. J. East, W. D. Ollis and R. E. Wheeler, forthcoming publication. T. J. Batterham and R. J. Highet, *Austral. J. Chem.* 17, 428 (1964).

TABLE 1. CHEMICAL SHIFTS (τ) FOR THE INDICATED PROTONS IN THE NMR SPECTRA OF DERIVATIVES OF LISETIN (XVa), PISCERYTHRONE (XXIIIa) AND PISCIDONE (XXVIa)

Location of protons or substituents	2-H [1]	6-H [1]	8-H [1]	3′-H [1]	6′-H [1]	4-OH [1]	OMe [3]	OAc [3]	$Me_2C{=}CH{-}CH_2{-}$				$Me_2C(O){-}CH_2{-}CH_2{-}$		
									Me* [3]	Me* [3]	H [1]	H_2 [2]	Me_2 [6]	H_2 [2]	H_2 [2]
Lisetin trimethyl ether (XVb)		3·59 (d, J = 2·5)	3·42 (d, J = 2·5)		2·42		6·15 6·15 6·08 6·02		8·30	8·17	4·68 (t, J = 7)	6·41 (d, J = 7)			
Lisetin triacetate (XVc)		3·08 (d, J = 2·5)	2·64 (d, J = 2.5)		2·48		6·10	7·67 7·67 7·52	8·30	8·19	4·78 (t, J = 7)	6·51 (d, J = 7)			
The 4-hydroxycoumarin (XVII)		3·82 (d, J = 2·5)	3·52 (d, J = 2·5)		3·25	0·18	6·23 6·20 6·15 6·02		8·33	8·22	4·77 (t, J = 7)	6·55 (d, J = 7)			
The 4-hydroxycoumarin (XVIII)		3·60 (d, J = 2·5)	3·43 (d, J = 2·5)		3·23	1·63	6·18 6·13 6·13 5·98						8·75	8·25 (t, J = 7)	7·22 (t, J = 7)
Isolisetin dimethyl ether (XVIb)		3·63 (d, J = 2·5)	3·47 (d, J = 2·5)		2·57		6·13 6·10 6·05						8·53	8·13 (t, J = 7)	7·05 (t, J = 7)
The 4-hydroxycoumarin (XIX)		3·54 (d, J = 2·5)	3·45 (d, J = 2·5)		3·18	0·08	6·18 6·09 5·95						8·56	8·16 (t, J = 7)	7·18 (t, J = 7)

Piscerythrone tetramethyl ether (XXIIIb)	2·22	3·72 (d, J = 2·5)	3·60 (d, J = 2·5)		3·20	6·57 6·23 6·22 6·18 6·12		8·35	8·25	4·85 (t, J = 7)	6·63 (d, J = 7)				
Piscerythrone tetracetate (XXIIIc)	2·15	3·10 (d, J = 2·5)	2·73 (d, J = 2·5)		3·23	6·22	7·92 7·73 7·70 7·62	8·30	8·29	4·95 (t, J = 7)	6·80 (d, J = 7)				
Piscidone tetramethyl ether (XXVIb)	2·46	3·62 (d, J = 2·5)	3·58 (d, J = 2·5)	3·53		6·22 6·15 6·12 6·12 6·12		8·52	8·42	5·00 (t, J = 7)	6·82 (d, J = 7)				
Piscidone tetracetate (XXVIc)	2·20	3·08 (d, J = 2·5)	2·70 (d, J = 2·5)	3·25		6·23	7·70 7·70 7·70 7·63	8·55	8·54	5·05 (t, J = 7)	6·90 (d, J = 7)				
Isopiscidone triacetate (XXVIIb)	2·27	3·22 (d, J = 2·5)	2·82 (d, J = 2·5)	3·70		6·27	7·72 7·72 7·65					8·72	8·32 (t, J = 7)	7·55 (t, J = 7)	

The spectra were determined in $CDCl_3$ solution with tetramethyl silane as an internal standard on a Varian A-60 spectrometer.

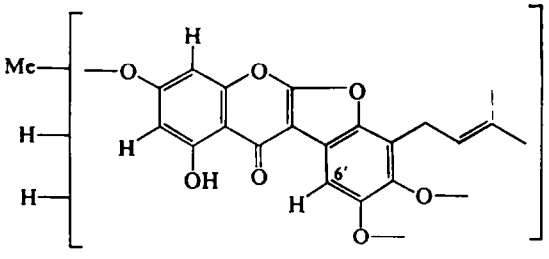
For ease of comparison of the chemical shifts of corresponding protons, the formulae of lisetin and its derivatives have been numbered to correspond with the usual convention for isoflavones.

Proton counts. The proton integrals are enclosed in square brackets and all signals had the appropriate integrated intensities.

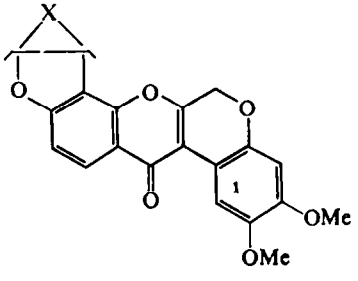
Multiplicities of signals. Unless otherwise indicated, all signals are singlets. For other cases d = doublet, t = triplet, and the coupling constants J are given in c/s.

* The methyl groups of the $\gamma\gamma$-dimethylallyl substituents are non-equivalent The high field methyl is *trans* to the vinyl hydrogen.[11]

Several natural products of the coumarono-coumarin type (cf. VIII) are known including coumestrol[13] (IX; $\nu_{CO} = 1700\ cm^{-1}$), wedelolactone[14] (X; $\nu_{CO} = 1707\ cm^{-1}$), erosnin[15] (XI; $\nu_{CO} = 1733\ cm^{-1}$) and psoralidin[16] (XII; $\nu_{CO} = 1701\ cm^{-1}$) and as indicated they all show carbonyl absorption in the range 1700–1740 cm^{-1}. Clearly the IR spectra of lisetin, its triacetate, and trimethyl ether are not in accord with the coumarono-coumarin type (VIII) so the alternative coumarono-chromone type (VII) was considered. This type of structure was attractive on several grounds. The relative positions of the carbonyl bands in lisetin ($\nu_{CO} = 1650\ cm^{-1}$), lisetin trimethyl ether ($\nu_{CO} = 1660\ cm^{-1}$) and lisetin triacetate ($\nu_{CO} = 1665\ cm^{-1}$) suggested that lisetin contained a carbonyl group of the chromone type, chelated with a hydroxyl group;[17] this was possible in a structure of the type VII. Furthermore, this structure provided an acceptable interpretation of the unusual chemical shift of the proton associated with the low field singlets in the NMR spectra of lisetin trimethyl ether ($\tau = 2{\cdot}42$) and lisetin triacetate ($\tau = 2{\cdot}48$). The selection of the partial structure VII for lisetin requires these singlets to be associated with a proton in either position 3′ or 6′ on ring B. Normally a proton in either of these positions on a trioxygenated benzene ring would have been shifted considerably upfield so clearly the proton must be specifically deshielded by the carbonyl function. This requires the proton in lisetin trimethyl ether (τ 2·42) and in lisetin triacetate (τ 2·48) to be placed in position 6′



XIII



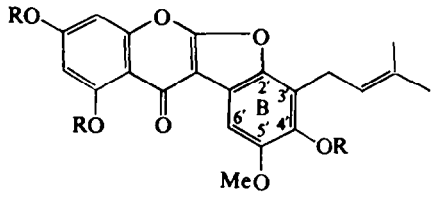
XIVa X = $Me_2C{-}CH{=}CH$

XIVb X = $Me_2CH{-}C{=}CH$

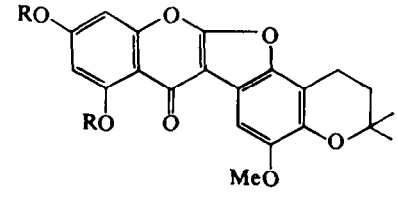
[13] E. M. Bickoff, R. L. Lyman, A. L. Livingstone and A. N. Booth, *J. Amer. Chem. Soc.* **80**, 3969 (1958).
[14] T. R. Govindachari, K. Nagarajan and B. R. Pai, *J. Chem. Soc.* 629 (1956), 545 (1957).
[15] J. Eisenbeiss and H. Schmid, *Helv. Chim. Acta* **42**, 61 (1959).
[16] H. N. Khastgir, P. C. Duttagupta and P. Sengupta, *Tetrahedron* **14**, 275 (1961).
[17] W. Baker, A. C. M. Finch, W. D. Ollis and K. W. Robinson, *J. Chem. Soc.* 1477 (1963).

(see VII) and this gives formula XIII as a favoured partial structure for lisetin. The deshielding of the proton in position 6′ (see XIII) is completely analogous to the corresponding deshielding shown by the protons in position 1 in 6a-12a-dehydrorotenoids,[18] such as 6a,12a-dehydrodeguelin (XIVa; H_1, τ 1·60) and 6a,12a-dehydroisorotenone (XIVb; H_1, τ 1·67).

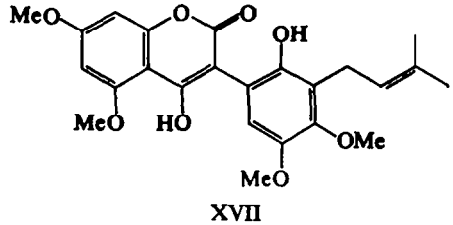
A detailed comparison (Table 1) of the chemical shifts of corresponding protons in lisetin triacetate and lisetin trimethyl ether now permitted the selection of a favoured position for the methoxyl group in lisetin. The AB system associated with the *meta*-related protons showed considerable differences in chemical shift in lisetin triacetate (τ 2·64 and 3·08; J = 2·5 c/s) and in lisetin trimethyl ether (τ 3·42 and 3·49; J = 2·5 c/s), whereas the singlet associated with the 6′-proton in lisetin triacetate (τ 2·48) and lisetin trimethyl ether (τ 2·42) shows only a slight difference. This suggested that the substituent in position 5′ must be the same in both the trimethyl ether and triacetate, and so it follows that the methoxyl group in lisetin is in position 5′ as in the formula XVa.



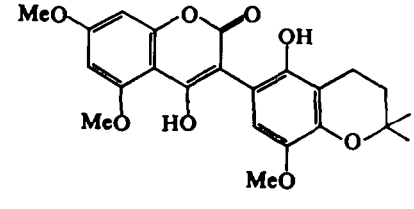
Lisetin* XVa R = H
XVb R = Me
XVc R = Ac



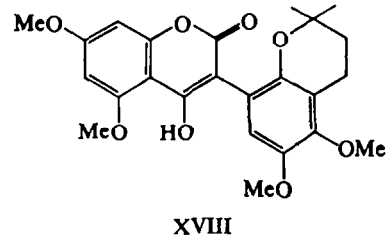
Isolisetin XVIa R = H
XVIb R = Me
XVIc R = Ac



XVII



XIX



XVIII

Although the structure XVa for lisetin was not fully established at this stage, it was used to design the following definitive experiments. Lisetin on mild treatment with acid was smoothly transformed into an isomer, isolisetin (XVIa), characterized as its

* For ease of comparison of chemical shifts of corresponding protons, the formula of lisetin and its derivatives have been numbered to correspond with the convention for isoflavones.

[18] L. Crombie and J. W. Lown, *J. Chem. Soc.* 775 (1962).

dimethyl ether (XVIb). The NMR spectrum of isolisetin dimethyl ether (Table 2) clearly indicated that an acid-catalysed addition of a phenolic hydroxyl group to the $\gamma\gamma$-dimethylallyl group had occurred to give a 2,2-dimethylchroman. The spectrum clearly showed a singlet due to a *gem*-dimethyl group ([6], τ 8·53) and a pair of triplets (J = 7 c/s) assigned to adjacent methylene groups ([2], τ 8·13 and [2], τ 7·05); the latter was clearly a benzylic methylene group.[2] The formation of isolisetin (XVIa) demonstrates the *ortho* relationship of the $\gamma\gamma$-dimethylallyl group and a phenolic hydroxyl group in lisetin (XVa) and in accord with the formula XVa for lisetin, the 6′-proton in lisetin trimethyl ether (XVb) and in isolisetin dimethyl ether (XVIb) both have very similar chemical shifts (τ 2·42 and 2·57 respectively).

Lisetin trimethyl ether (XVb) is hydrolysed by dilute alkali to give a product which is clearly the 4-hydroxycoumarin (XVII) since it shows a carbonyl band at ν_{CO} = 1710 cm^{-1} characteristic of coumarins. When the 4-hydroxycoumarin (XVII) was treated with acid, cyclization occurred to give the isomeric 4-hydroxycoumarin (XVIII). It may be noted that two paths of reaction are, in principle, available to the compound XVII on treatment with acid, either cyclization or dehydration, and as expected the former reaction might be predicted to occur preferentially. Similarly, when isolisetin dimethyl ether (XVIb) is treated with alkali, then it also yields a 4-hydroxycoumarin (XIX). The development of the structure XVa for lisetin initially involved the adoption for biogenetic reasons of the oxygenation pattern of the partial structure VI. However, the reaction sequences XVb → XVII → XVIII and XVa → XVIa → XVIb → XIX prove that the ethereal oxygen, the $\gamma\gamma$-dimethylallyl group, and the hydroxyl group must be located in positions 2′, 3′ and 4′ respectively. The 6′-position of the hydrogen on ring B was already established so the methoxyl group of lisetin must be placed in position 5′. As expected, the deshielding of the proton in position 6′, which is a characteristic of derivatives of lisetin is not apparent in the NMR spectra of the 4-hydroxycoumarins (XVII, XVIII and XIX; Table 1). In the latter compounds, the 6′-proton is no longer constrained to lie in the deshielding cone of the carbonyl group.[18]

The formation of the 4-hydroxycoumarins (XVII and XIX) is mechanistically analogous to the alkaline hydrolysis of isoflavones to give eventually deoxybenzoins and formic acid and this clearly demonstrates that lisetin is a coumarono-chromone (VII) and is not a coumarono-coumarin (VIII) since compounds of the latter type[13–16] just give hydroxy-acids on alkaline hydrolysis. Thus lisetin represents a new variant upon the isoflavonoid theme[19] and the formation of 4-hydroxycoumarins by alkaline hydrolysis may be useful in the recognition of this new type of natural product.

The final step in the argument concerning the structure of lisetin is provided by its partial synthesis from piscerythrone. This is discussed in the sequel.

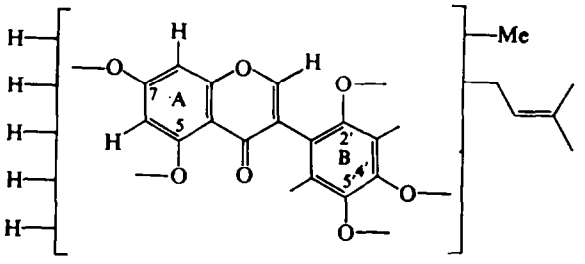
The constitutions of piscerythrone and piscidone

In addition to lisetin, a number of other compounds was isolated from the root of *P. erythrina* including two new natural products which have been named piscerythrone and piscidone.

Piscerythrone and piscidone are isomers and comparison of their molecular formula, $C_{20}H_{17}O_6(OMe)$, with that of lisetin, $C_{20}H_{15}O_6(OMe)$, suggested a close structural relationship between these three compounds. Piscerythrone and piscidone

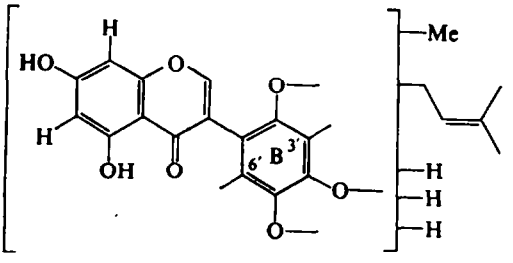
[19] W. D. Ollis, *The Chemistry of Flavonoid Compounds* (Edited by T. A. Geissman) p. 353. Pergamon, Oxford (1961).

each formed tetracetates and tetramethyl ethers, but the fact that the tetramethyl ethers were different required some skeletal differences. The IR spectra of piscerythrone and piscidone and their derivatives showed many common features. They also showed similar UV spectra (Table 2) and these UV spectral characteristics in association with a probable relationship to lisetin (XVa) suggested that piscerythrone and piscidone were isoflavones. The NMR spectra of the tetracetates and tetramethyl ethers of piscerythrone and piscidone (Table 1) showed features compatible with the partial structure XX. The presence of a $\gamma\gamma$-dimethylallyl group as an aromatic substituent was clearly indicated[11] and the AB system characteristic of *meta*-related protons on a phloroglucinol-type ring[12] could be recognized in all spectra. On this evidence, the oxygenation pattern shown in the partial structure XX was favoured; as far as ring B was concerned, the 2′,4′,5′-oxygenation pattern is characteristic of many natural isoflavones[19] including jamaicin (I) and ichthynone (II) also isolated from this plant.



XX

The magnitude of the downfield shift of the *meta*-related protons on ring A in piscerythrone tetracetate (τ 3·10 and 2·73; J = 2·5 c/s) and piscidone tetracetate τ 3·08 and 2·70; J = 2·5 c/s) as compared with piscerythrone tetramethyl ether (τ 3·72 and 3·60; J = 2·5 c/s) and piscidone tetramethyl ether (τ 3·62 and 3·58; J = 2·5 c/s) showed that the substituents in positions 5 and 7 (see XX) were both hydroxyl, thus giving the partial formula XXI for piscerythrone and piscidone.



XXI

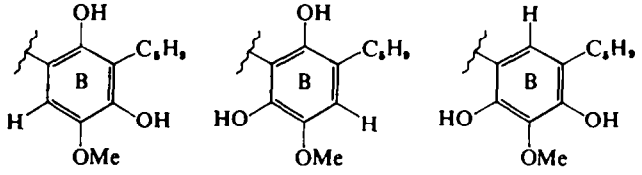
Since piscerythrone and piscidone gave different tetramethyl ethers, this required that the major structural difference between them involved the position of the $\gamma\gamma$-dimethylallyl substituent on ring B. In one compound, the $\gamma\gamma$-dimethylallyl group could be in position 3′ and in the other in position 6′. A decision involving these possibilities has been made on the following grounds. The chemical shift of the aromatic proton on ring B in piscerythrone tetracetate and piscerythrone tetramethyl ether (τ 3·23 and 3·20 respectively) were so similar that it was probable that the same group of substituents was located either *ortho* or *para* to this proton in both

TABLE 2. UV SPECTRA OF LISETIN (XVa), PISCERYTHRONE (XXIIIa), PISCIDONE (XXVIa), AND THEIR DERIVATIVES
λ_{max} mμ (ε_{max}) in ethanol

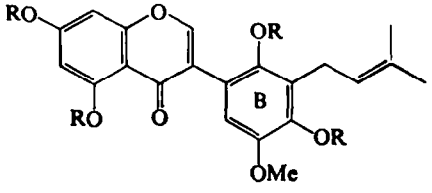
Coumarano-chromone derivatives					
Lisetin (XVa)	258	(39,400)	284	(23,200)	338 (13,900)
Lisetin trimethyl ether (XVb)	259	(32,600)	277	(17,800)	320 (15,100)
Lisetin triacetate (XVc)*	246	(28,320)	273 inf	(18,710)	298 (8,820)
					317 (11,270)
Isolisetin (XVIa)	260	(35,910)	285	(20,270)	337 (12,470)
Isolisetin dimethyl ether (XVIb)	259	(33,610)	272 sh	(20,330)	307 (14,350)
					327 (15,000)
4-Hydroxycoumarin derivatives					
(XVII)					320 (16,230)
(XVIII)					325 (20,150)
(XIX)					320 (15,360)
Isoflavone derivatives					
Piscerythrone (XXIIIa)	265	(23,700)	294	(14,900)	
Piscerythrone tetramethyl ether (XXIIIb)	255	(31,060)	285	(13,820)	
Piscerythrone tetracetate (XXIIIc)	243	(27,670)	286	(11,880)	
Piscidone (XXVIa)	260	(28,470)	296	(8,210)	
Piscidone tetramethyl ether (XXVIb)	251	(27,820)	280 inf	(10,270)	
Piscidone tetracetate (XXVIc)	245 inf	(24,250)	282	(8,570)	
Isopiscidone (XXVIIa)	262	(28,600)	300 sh	(8,480)	
Isopiscidone triacetate (XXVIIb)			285 inf	(7,500)	

* Solvent: dioxan

derivatives. This condition gives three possible arrangements of substituents on ring B in piscerythrone and of these XXIIa, XXIIb, and XXIIc, only one, namely XXIIa, is compatible with the partial formula XXI.

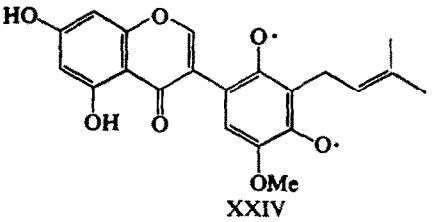


XXIIa XXIIb XXIIc

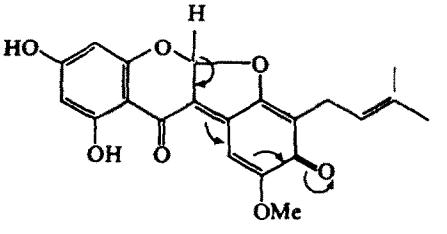


Piscerythrone XXIIIa R = H
XXIIIb R = Me
XXIIIc R = Ac

Thus XXIIIa was the favoured structure for piscerythrone and this was confirmed by the direct transformation of piscerythrone into lisetin (XVa) by oxidation with alkaline potassium ferricyanide. Although further work is required[20] to establish the mechanistic details of this partial synthesis of lisetin (XVa) from piscerythrone (XXIIIa) there is ample precedent[21] for believing that this reaction proceeds by a radical coupling process involving for example the biradical XXIV which via XXV could yield lisetin (XVa).



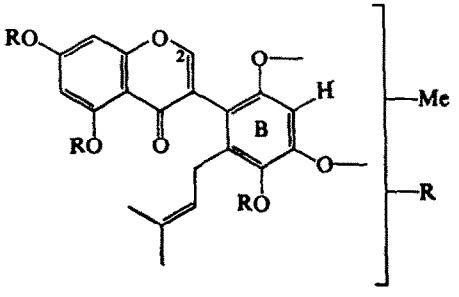
XXIV

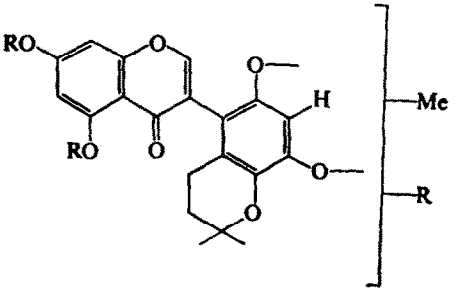


XXV

The oxidative transformation of piscerythrone to lisetin permits an interpenetration of the independent arguments leading to a deduction of their structures and, although degradations to known compounds have not been carried out, the structures proposed for lisetin (XVa) and piscerythrone (XXIIIa) are considered to be fully established.

If piscidone and piscerythrone are both represented by the partial formula XXI then the fact that they give different tetramethyl ethers places the $\gamma\gamma$-dimethylallyl substituent in piscidone in position 6'. Acid-catalysed isomerization of piscidone gave isopiscidone characterized as its triacetate and the NMR spectrum of isopiscidone triacetate (Table 1) clearly showed that cyclization to form a 2,2-dimethylchroman ring had occurred.* It follows that a hydroxyl is present in piscidone in position 5' and this leads to two possible structures (XXVIa) for piscidone. The corresponding two structures (XXVIIa) for isopiscidone follow.





Piscidone XXVIa R = H XXVIb R = Me XXVIc R = Ac

Isopiscidone XXVIIa X = H XXVIIb R = Ac

* Under the same conditions piscerythrone (XXIIIa) gave a mixture which could not be separated. Presumably the product was a mixture of isomeric 2,2-dimethylchromans which may be formed because two phenolic hydroxyls are located *ortho* to the $\gamma\gamma$-dimethylallyl group.

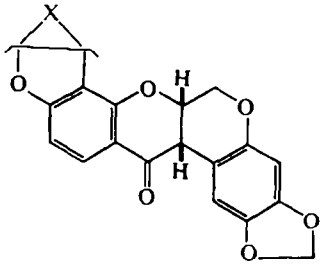
[20] C. A. Rhodes and W. D. Ollis, forthcoming publication.

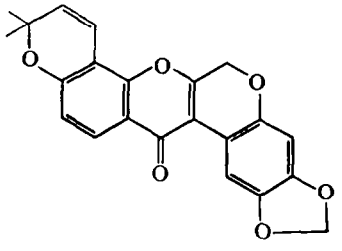
[21] D. H. R. Barton and T. Cohen, *Festschrift Arthur Stoll* p. 117. Birkhäuser, Basel (1957); H. Erdtman and C. A. Wachtmeister, *Festschrift Arthur Stoll* p. 144. Birkhäuser, Basel (1957); C. H. Hassall and A. I. Scott, *Recent Developments in the Chemistry of Natural Phenolic Compounds* (Edited by W. D. Ollis) p. 119. Pergamon, Oxford (1961); A. R. Battersby, *Proc. Chem. Soc.* 189 (1963); D. H. R. Barton, *Ibid.* 293 (1963); A. I. Scott, *Quart. Revs.* **19**, 1 (1965).

Further work to discriminate between the two possible structures (XXVIa) for piscidone is in progress. It may be noted, however, that piscidone is an unusual isoflavone in that it is 2',6'-disubstituted and this may be associated with an unusual feature in the NMR spectra of piscidone derivatives. The singlet associated with the proton in position 2 in these compounds occurs at unusually high field and this is particularly noticeable in piscidone tetramethyl ether (2-H; τ 2·46). It is probable that the unusual shielding experienced in this case is due to the steric situation associated with the 2'- and 6'-substituents. This could cause ring B to be twisted out of the plane which approximately accommodates the chromone part of the structure and in this conformation the proton in position 2 would be in the positive shielding zone of the aromatic ring B.

The rotenoids from P. erythrina

In addition to the isoflavonoids (I, II, XVa, XXIIIa, and XXVIa), several rotenoids have been isolated including rotenone (III) and sumatrol (IV). Sumatrol was recognized because its structure could be deduced from its NMR spectrum and comparison with the published NMR spectral characteristics of sumatrol[18] confirmed its identity.





Millettone XXVIIIa X = $Me_2C{-}CH{=}CH$

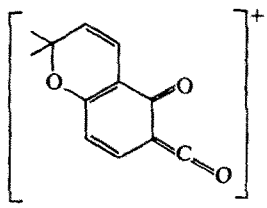
XXIX

Isomilletone XXVIIIb X = $CH_2{=}\underset{\displaystyle Me}{C}{-}CH{-}CH_2$

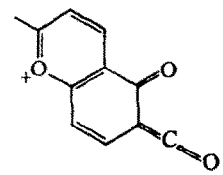
A substance was isolated and its NMR spectrum indicated that it was a mixture of two compounds. All attempts to resolve this mixture have so far failed, but its NMR spectrum had many features in common with the spectrum of millettone (XXVIIIa), which we first isolated from *Millettia dura*.[9] Complete coincidence in the NMR spectra of the signals due to ten protons indicated that both components of this mixture had the same rotenoid skeleton (see XXVIII) and the reduced intensity of the signals due to the 2,2-dimethylchromene residue in millettone (XXVIIIa) was exactly compensated by signals of the appropriate intensity to be associated with a residue of type X in the formula XXVIIIb. We regard this as evidence that the other component of this mixture is isomillettone (XXCIIIb) which is a new rotenoid with the same type of isoprenoid residue as occurs in rotenone (III) and sumatrol (IV).

A yellow compound was also isolated in very small amount. Its mass spectrum showed a strong molecular ion peak at *m/e* 376, an (M—CH_3) peak at *m/e* 361, and a doubly charged species corresponding to this ion at *m/e* 180·5. The mass spectrum also showed significant peaks at *m/e* 202, 187 and 173. The interpretation of this mass spectrum was easily possible when it was considered in relation to the mass spectra of

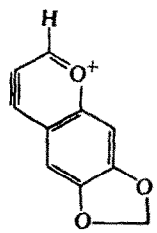
several other natural products. Thus millettone (XXVIIIa) and jamaicin (I), which are isomers with the molecular formula $C_{22}H_{18}O_6$, show parent peaks at *m/e* 378 and strong ions at *m/e* 202 and 187. Thus the ions *m/e* 202, 187 and 173 observed in the mass spectrum of the yellow compound may be assigned the structures XXX, XXXI and XXXII respectively.



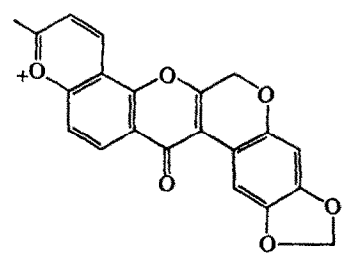
XXX *m/e* 202



XXXI *m/e* 187



XXXII *m/e* 173



XXXIII *m/e* 361; *m/2e* 180·5

A possible structure for the yellow compound was dehydromillettone (XXIX; $C_{22}H_{16}O_6$) and the loss of a methyl group from the 2,2-dimethylchromene ring to give the ions XXXI and XXXIII has been recognized[22] as a favoured fragmentation process. The identification of dehydromillettone (XXIX) was confirmed by comparison with an authentic specimen.[9] Previously there has been some uncertainty about the existence of dehydrorotenoids as natural products,[23] but we believe that dehydrodeguelin (XIVa) from *Millettia dura*[9] and dehydromillettone (XXIX) from *P. erythrina* are not artefacts but that they are natural products.

Piscidia erythrina has been shown to be a particularly good example for illustrating the natural co-occurrence[23] of isoflavonoids and rotenoids. The compounds isolated from this source include the isoflavones, jamaicin (I), ichthynone (II), piscerythrone (XXIIIa), and piscidone (XXVIa), the rotenoids, rotenone (III), sumatrol (IV), millettone (XXVIIIa), isomillettone (XXVIIIb), and dehydromillettone (XXIX), and lisetin (XVa) which is representative of a new structural type among the isoflavonoids. Since this study was completed, lisetin has also been isolated from *Ichthyomethia communis*.[24]

The optical rotatory dispersion curves of rotenone (III), sumatrol (IV), millettone (XXVIIIa), and isomillettone (XXVIIIb) isolated from *P. erythrina* establish that these compounds all have the same absolute configurations (6aS, 12aS, 5'R) characteristic of natural rotenoids.[8c]

[22] C. S. Barnes and J. L. Occolowitz, *Austral. J. Chem.* **17**, 975 (1964); B. Willhalm, A. F. Thomas and F. Gautschi, *Tetrahedron* **20**, 1185 (1964).

[23] H. Grisebach and W. D. Ollis, *Experientia* **11**, 1 (1961).

[24] E. R. Crossley, C. P. Falshaw and W. D. Ollis, forthcoming publication.

EXPERIMENTAL

M.ps were taken on a Kofler block and are uncorrected. The silica gel used in column chromatography was the Hopkins and Williams' Grade M.F.C., and Kieselgel G (Merck) was used in TLC.

Extraction of the total root of Piscidia erythrina *L.* The total root material used in this investigation was freshly collected in Jamaica. It was dried, finely ground, and the material (2400 g) was continuously extracted during 24 hr with hot light petroleum (b.p. 60–80°). This extract on keeping at 0° yielded a solid *A* (3·0 g) which was collected. Evaporation of the filtrate gave a residue *B* (28 g).

The root material was then continuously extracted during 48 hr with hot ether and this extract on evaporation gave a residue *C* (168 g).

Examination of fraction A

Isolation of jamaicin (I) *and rotenone* (III). Fractional crystallization of solid *A* (3·0 g) from EtOH gave jamaicin (1·1 g) as the less soluble fraction and rotenone (850 mg). The jamaicin separated from EtOH as characteristic colourless prisms, m.p. 193–194° (lit.[5] m.p. 193–194°), and the rotenone as white prisms, m.p. 163–164° (lit.[25] m.p. 163–164°). Direct comparison (mixed m.p., UV, IR, and NMR spectra) of these two compounds with authentic specimens established their identity.

Examination of fraction B

Isolation of millettone (XXVIIIa), *isomillettone* (XXVIIIb), *dehydromillettone* (XXIX), *sumatrol* (IV), *rotenone* (III), *jamaicin* (I), *ichthynone* (II) *and PE* 8. A solution of the residue *B* (28 g) in benzene (300 ml) was fractionated chromatographically using a silica gel column (1100 g). Elution with benzene (fractions 1–35), benzene–chloroform mixtures (fractions 36–67), and chloroform (fractions 68–80) gave eighty 2 l. fractions. The IR spectrum of a sample of each fraction was determined and an indication of the composition of each fraction was examined by TLC. Where appropriate, similar fractions were combined and further chromatography and fractional crystallization gave the following results:

Fractions 1–3. Fatty materials which were not further examined.

Fractions 4–6. These yielded a mixture (3 g) of millettone and isomillettone as colourless needles, m.p. 174–179°, from chloroform–MeOH.

Fraction 7. Dehydromillettone (2·5 mg; m.p. 358° dec).

Fractions 8–14. No major components isolated.

Fractions 15–16. These yielded sumatrol (70 mg) as white needles, m.p. 194–196° (lit.[8] m.p. 195–197°).

Fractions 17–20. These yielded rotenone (1·4 g), m.p. 162–164° from EtOH.

Fractions 21–27. These yielded mixtures (3·3 g) of rotenone and jamaicin.

Fractions 28–39. No major components isolated.

Fractions 40–41. These yielded ichthynone (1·2 g), m.p. 203–204° (lit.[9] m.p. 202·5–203·5°).

Fractions 42–49. These yielded mixtures of ichthynone and an unidentified compound PE 8 (32 mg), m.p. 264–270°.

Fractions 50–80. No major components isolated.

Examination of fraction C

Isolation of piscidone (XXVIa), *piscerythrone* (XXIIIa), *and lisetin* (XVa). A portion (89 g) of fraction *C* was dissolved in benzene (300 ml) and after standing overnight a buff-coloured solid (1·2 g) separated. This was collected and recrystallized from aqueous EtOH giving *piscidone* (500 mg) as cream needles, m.p. 154–155°. λ_{max} (EtOH) 260 mμ (ε 28,470), 296 mμ (ε 8,210); ν_{max} (nujol) 1655 cm^{-1}. (Found: C, 65·35; H, 5·14. $C_{21}H_{20}O_7$ requires: C, 65·61; H, 5·24%.)

After collecting the crude piscidone, the benzene filtrate was then chromatographed on a silica gel column (1300 g). Two-litre fractions were collected by successive elution with benzene (fractions 1–14), benzene–chloroform (3:1 v/v, fractions 15–32), benzene–chloroform (1:1 v/v, fractions 33–59), and chloroform (fractions 60–80). Only fractions 51–59 were rewarding and they yielded crystalline solids on trituration with ether–light petroleum. These were combined and fractional crystallization from ether gave *piscerythrone* (600 mg) as yellow needles, m.p. 183·5–184·5°. λ_{max} (EtOH) 265 mμ

[25] A. Butenandt and F. Hildebrandt, *Liebigs Ann.* **477**, 245 (1929).

(ε 23,700), 294 mμ (ε 14,900); ν_{max} ($CHCl_3$) 3600, 1655 cm^{-1}. (Found: C, 65·87; H, 5·70. $C_{21}H_{20}O_7$ requires: C, 65·61; H, 5·24%.)

The ethereal mother liquors from the crystallization of piscerythrone were evaporated and crystallization of the residue (403 mg) from acetone gave lisetin as colourless cubes, m.p. 283–286° dec, identical (mixed m.p., UV and IR spectra) with an authentic specimen (lit.[3] m.p. 284–285° dec. λ_{max} (EtOH) 258 mμ (ε 39,400), 284 mμ (ε 23,200), 338 mμ (13,900); ν_{max} ($CHCl_3$) 1653 cm^{-1} (Found: C, 66·17; H, 5·40; OMe, 7·58. $C_{30}H_{18}O_6$ (OMe) requires: C, 65·96; H, 4·75; OMe, 8·12%.)

Lisetin triacetate (XVc). A mixture of lisetin (100 mg), acetic anhydride (1 ml), and anhydrous pyridine (1 ml) was kept at room temp for 24 hr and then poured into water. The solid was collected and recrystallization from MeOH gave lisetin triacetate (80 mg) as colourless needles, m.p. 254–255°. λ_{max} (dioxan) 246 mμ (ε 28,320), 273 mμ inf. (ε 18,700), 298 mμ (ε 8,820), 317 mμ (ε 11,270); ν_{max} ($CHCl_3$) 1770, 1665, 1630 cm^{-1}. (Found: C, 63·79; H, 4·75. $C_{27}H_{24}O_{10}$ requires: C, 63·77; H, 4·76%.)

Lisetin trimethyl ether (XVb). A mixture of lisetin (150 mg) MeI (5 ml), anhydrous K_2CO_3 (1·5 g), and acetone (50 ml) was heated under reflux for 18 hr, filtered, and the filtrate evaporated. The residue was extracted with hot chloroform (2 × 20 ml) and this yielded *lisetin trimethyl ether* (124 mg) as white needles, m.p. 199–200°, from MeOH. λ_{max} (EtOH) 259 mμ (ε 32,600), 277 mμ (ε 17,800), 320 mμ (ε 15,100); ν_{max} ($CHCl_3$) 1660 cm^{-1}. (Found: C, 67·60; H, 5·72. $C_{24}H_{24}O_7$ requires: C, 67·91; H, 5·78%.)

Alkaline hydrolysis of lisetin trimethyl ether (XVb)

Formation of the 4-hydroxycoumarin (XVII). A mixture of lisetin trimethyl ether (254 mg), 10% KOHaq (20 ml), and MeOH (25 ml) was heated (N_2 atm) on a steam bath for 2 hr, cooled, acidified (2N HCl) and extracted with ether. This extract, after washing and drying ($MgSO_4$), yielded the *4-hydroxy-coumarin* (XVII; 152 mg) as stout white needles, m.p. 152–153°, from aqueous EtOH. λ_{max} (EtOH) 320 mμ (ε 16,230); ν_{max} 1710 and 1610 cm^{-1}. (Found: C, 65·45; H, 5·70. $C_{24}H_{26}O_8$ requires: C, 65·15; H, 5·90%.)

Acid-catalysed cyclization of the 4-hydroxycoumarin (XVII)

Formation of the isomer (XVIII). The 4-hydroxycoumarin (XVII; 46 mg), glacial AcOH (5 ml), and conc H_2SO_4 (1 ml) were warmed to 60° during 15 min, cooled and poured into water. The precipitate was collected and crystallized from aqueous MeOH giving the *product* (XVIII) as colourless prisms, m.p. 167–170°. λ_{max} (EtOH) 325 mμ (ε 20,150); ν_{max} ($CHCl_3$) 1710, 1650, and 1620 cm^{-1}. (Found: M (high resolution mass spectrometry), 442·1651. $C_{24}H_{26}O_8$ requires: M, 442·1628.)

Acid-catalysed isomerization of lisetin

Formation of isolisetin (XVIa). A mixture of lisetin (195 mg), glacial AcOH (20 ml), and conc H_2SO_4 (5 ml) was warmed to 60° after 15 min the lisetin had dissolved. The orange solution was then poured into water, the solid collected and recrystallized from aqueous MeOH giving *isolisetin* (87 mg) as colourless needles, m.p. 287–290° dec, λ_{max} (EtOH) 260 mμ (ε 35,900), 285 mμ (ε 20,270), and 337 mμ (ε 12,470); ν_{max} ($CHCl_3$) 1650 cm^{-1}. This material analysed as a *monohydrate*. (Found: C, 63·14; H, 4·95. $C_{21}H_{18}O_7 \cdot H_2O$ requires: C, 62·99; H, 5·04%), but the anhydrous compound was obtained by vigorous drying (100°/18 hr/0·5 mm.) (Found: C, 65·75; H, 4·97. $C_{21}H_{18}O_7$ requires: C, 65·96; H, 4·75%.)

Isolisetin dimethyl ether (XVIb). Methylation of isolisetin as for the preparation of lisetin trimethyl ether (see above) gave *isolisetin dimethyl ether*, m.p. 268–270°, from MeOH, λ_{max} (EtOH) 235 mμ sh (ε 25,840), 259 mμ (ε 33,610), 272 mμ sh (ε, 29,330), 307 mμ (ε 14,350), 327 mμ (ε 15,000); ν_{max} ($CHCl_3$) 1660 cm^{-1}. (Found: C, 67·00; H, 5·43. $C_{23}H_{22}O_7$ requires: C, 67·31; H, 5·40%.)

Alkaline hydrolysis of isolisetin dimethyl ether (XVIb)

Formation of the 4-hydroxycoumarin (XIX). Isolisetin dimethyl ether (68 mg) was hydrolysed as for lisetin trimethyl ether (see above) giving the 4-hydroxycoumarin (30 mg) as pale yellow prisms, m.p. 159–161°, from aqueous MeOH, λ_{max} (EtOH) 329 mμ (ε 15,360); ν_{max} 1700, 1660, and 1610 cm^{-1}. (Found: M (high resolution mass spectrometry), 428·1480. $C_{22}H_{24}O_8$ requires: M, 428·1471.)

Piscidone tetracetate (XXVIc). Piscidone (94 mg) was dissolved by gentle warming in a mixture

of Ac_2O (0·5 ml) and pyridine (0·5 ml) and after standing at room temp for 30 hr, the mixture was poured into water. The precipitate was collected and recrystallized from aqueous MeOH to give *piscidone tetracetate* as colourless prisms (54 mg), m.p. 148–150°, λ_{max} (EtOH) 245 mμ inf (ε 24,250), 282 mμ (ε 8,570); ν_{max} ($CHCl_3$) 1775, 1660, 1640 cm^{-1}. (Found: C, 62·56; H, 5·08. $C_{29}H_{28}O_{11}$ requires: C, 63·08; H, 5·11%.)

Piscerythrone tetracetate (XXIIIc). As in the preceding experiment, piscerythrone was characterized as its *tetracetate*, colourless prisms, m.p. 146–147°, from aqueous MeOH, λ_{max} (EtOH) 243 mμ (ε 27,670), 286 mμ (ε 11,880); ν_{max} ($CHCl_3$) 1770, 1660, 1640 cm^{-1}. (Found: C, 63·24; H, 4·95. $C_{29}H_{28}O_{11}$ requires: C, 63·08; H, 5·11%.)

Piscidone tetramethyl ether (XXVIb). A mixture of piscidone (160 mg), MeI (5 ml), and anhydrous K_2CO_3 (750 mg) in acetone (30 ml) was heated under reflux for 18 hr, cooled and filtered. The filtrate gave a residue which was extracted with warm chloroform yielding *piscidone tetramethyl ether* (86 mg) as white needles, m.p. 134–135°, from MeOH, λ_{max} (EtOH) 251 mμ (ε 27,820), 280 mμ inf (ε 10,270); ν_{max} ($CHCl_3$) 1660, 1615 cm^{-1}. (Found: C, 68·06; H, 6·32. $C_{25}H_{28}O_7$ requires: C, 68·17; H, 6·41%.)

Piscerythrone tetramethyl ether (XXIIIb). As in the preceding experiment, piscerythrone was characterized as its tetramethyl ether, colourless prisms, m.p. 174–175°, from aqueous MeOH, λ_{max} (EtOH) 255 mμ (ε 31,060), 285 mμ (ε 13,820); ν_{max} ($CHCl_3$) 1650, 1610 cm^{-1}. (Found: C, 68·44; H, 6·50. $C_{25}H_{28}O_7$ requires: C, 68·17; H, 6·41%.)

Acid-catalysed isomerization of piscidone (XXVIa)

Formation of isopiscidone (XXVII). A mixture of piscidone (230 mg), glacial AcOH (12 ml), and conc H_2SO_4 (4 ml) was warmed to 60° and after 15 min the piscidone had completely dissolved. The orange solution was poured into water (70 ml), the solid collected and crystallized from EtOH giving *isopiscidone* (74 mg) as white microcrystals, m.p. 163–165°, λ_{max} (EtOH) 262 mμ (ε 28,600), 300 mμ sh (ε 8,480); ν_{max} ($CHCl_3$) 1655 cm^{-1}. (Found: C, 65·27; H, 5·59. $C_{21}H_{20}O_7$ requires: C, 65·61; H, 5·24%.)

Isopiscidone triacetate (XXVIIb). Acetylation of isopiscidone as for piscidone (see above) gave *isopiscidone triacetate* as colourless prisms, m.p. 145–147°, from aqueous MeOH. λ_{max} (EtOH) 285 mμ inf (ε 7,500); ν_{max} ($CHCl_3$) 1755 1655, 1630 cm^{-1}. (Found: C, 63·68; H, 5·44. $C_{27}H_{26}O_{10}$ requires: C, 63·52; H, 5·13%.)

Oxidation of piscerythrone (XXIIIa) *to lisetin* (XVa). Potassium ferricyanide (235 mg, 1·36 molar equiv) was added to a solution of piscerythrone (202 mg) and K_2CO_3 (500 mg) in 50% aqueous MeOH (20 ml) at room temp (N_2 atm). After 10 min, the dark-coloured solution was acidified and the AcOEt extract after washing and drying ($MgSO_4$) yielded a buff-coloured solid. Crystallization from acetone gave lisetin (71 mg) identical (m.p. and mixed m.p. 285–286°; IR spectra) with natural lisetin.

The partially synthetic lisetin gave on methylation a product identical (m.p. and mixed m.p. 199–200°; IR spectra) with lisetin trimethyl ether.