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THE FLAVONES OF *APULEIA LEIOCARPA**†

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Abstract-The wood of *Apuleia leiocarpa* (Vog.) Macbr. (Leguminosae, subfamily Caesalpinioideae) yielded (+)-pinitol, β-sitosterol and ten flavones. Three of these flavones are the known compounds ayanin (X), oxyayanin-A (VIIIa) and oxyayanin-B (IX). The constitutions of the seven new flavones, apulein (Ia), 5-*O*-demethylapulein (Ib), apuleisin (Va), apuleitrin (VIa), apuleirin (VIe), apuleidin (VIIa) and 5-*O*-methyl-oxyayanin-A (VIIIb) were established. A structural proposal is also advanced for leiocarpin (XVI), a new pterocarpan isolated from the bark. The unusual oxygenation pattern of the flavones is discussed.

INTRODUCTION

Apuleia leiocurpa (Vog.) Macbr. (= A. *praecox* Mart.) (Leguminosae-Caesalpinioideae) is a tree which may attain considerable proportions. The fine-textured durable yellow timber is highly appreciated for heavy construction, flooring, door frames, wheel-wright work, shafts of vehicles and fence posts.' The species occurs over a huge territory from Northern Argentine. Ducke cites also A. *molaris* Spr. ex Bth, which grows in the Amazon valley.2 More recently, however, the opinion prevails that the genus *Apuleia* Mart. is monotypic and that *A. leiocarpa* is the binomial to be retained.[3] We have compared, by TLC, extracts of different parts of specimens of *A. molaris*, collected near Belém, Pará State, and classified by the botanist J. Murça Pires, and of *A. leiocarpa*, collected near Brasilia, D. F., and classified by the botanist E. P. Heringer, and were unable to detect any difference in chemical composition.

The trunk of the last mentioned specimen was separated into heartwood, sapwood and bark, which were extracted separately. By a combination of fractional crystallization and column chromatography (+)-pinitol, β-sitosterol and nine flavones were isolated from the heartwood extract. A tenth flavone was shown to be present by TLC. The same flavones and β-sitosterol were also detected in the sapwood extract. The composition of the bark extract, however, was found to be different. Instead of the flavones, a new pterocarpan and β-sitosterol were present.

The derivation from a flavone skeleton of the group of nine heartwood constituents was immediately apparent upon inspection of their UV spectra. In order to classify the substances

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[1] W. B. MORS and C. T. RIZZINI, *Useful Plants of Brazil*, p. 124, Holden-Day, San Francisco (1966).

[2] A. DUCKE, Notas sôbre a Flora Neotrópica. II. As Leguminosas da Amazonia Brasileira, 2nd edition, p. 112, *Boletim Téchnico do Instituto Agronômico do Norte, No.* 18, Belém, Brasil (1949).

[3] C. T. RIZZINI, Jardim Botânico do Rio de Janeiro, personal communication (1969); see also S. J. RECORD and P. W. HESS, *Timbers of the New World*, p. 233, Yale University Press, New Haven (1943).

TABLE 1. NMR SPECTRA OF *Apukia*

Compound	MHz	6-H	8-H	2'-H	3'-H	5'-H	6'-H	5-OH	2'-OH	OH	OH
Ia	100		3.26	—	3·40	—	2·77	—	2·00	4.55	
Ib	100	—	3·47	—	3.37	—	2·74	-2.28	2.13	4.67	
Ic	100	—	3.36	—	3.18	—	2.69	—	—		
Id	60	—	3.15	—	3.08	—	2.57	—	—	—	
Ie	60		3.16	—	3·14	—	2·67	—		—	
Va	60	—	3.42	—	—	3.26 d *J*8·4	2·76 d *J*8·4	-2.24	2·02	4.56	4·16
Vb	60	—	3.33	—	—	3.23 d *J*8·4	2.86 d *J*8·4	—	—	—	
vc	60	—	3.20	—	—	2.98 d *J*8·6	2.47 d *J*8·6	—	—	—	
Vd	60	—	3.42	—	—	3.28 d *J*9·0	2·10 to 2·76*	-2.53	—	4.52	
Ve	60	—	3·52	—	—	3.19 d *J*8·5	2.79 d *J*8·5	—2·72	—	—	
Vf	60	—	3.18	—	—	3.20 d *J*8·5	2.82 d *J*8·5	—	—	—	
VIa	60	—	3.45	264	—	—	2.64	-243	—	4·35	3·70
VId	60	—	3.08	2.32 d *J*2·3	—	—	2·55 d J2.3	—	—	—	
VIe	60	—	3.15	2.55	—	—	2.55	—	—	4·20	3·90
v u	60	—	3.25	2.33 d *J*2·1	—	—	2.61 d 2·1	—	—	—	
VIIa	60	3.62 d *J*2·3	3.54 d J2.3	—	—	3.27 d *J*8·7	2·73 d *J*8·7	-2.46	1.95	—	4·05
VIIb	60	3.35 d *J*2·3	3.25 d J2.3	—	—	2.96 d *J*8·7	2.42 d *J*8·7	—	—	—	
VIIIa	100	3.63 d *J*2	3.56 d J2	—	3.41	—	2.73	-2.35	2.14	4.72	
VIIId	60	3.63 d *J*2·1	3.56 d *J*2·1	—	3.13	—	2.58	—	—	—	
VIIIe	60	3.26 d J2.6	3.41 d J2.6	—	3.16	—	2.65	—	—	—	
X	60	3.68 d J2	3.56 d J2	2.32 d J2	—	3·02 d J 9	2·27 dd *J*2 & 9	-2.86	—	—	4·22

* m, includes the 10-H-signal of the two phenyl groups.

† Unless otherwise indicated, the chemical shift values (τ) refer to singlets. d ... doublet; dd ... double doublet. Identical values for two or more signals indicate overlap of the corresponding spectral lines. When localization of groups is not specified, individualization of signals was either doubtful, or omitted to allow inclusion of values for differently located groups under the same heading.

CONSTITUENTS AND OF THEIR DERIVATIVES†

OMe	OMe	OMe	OMe	OMe	OMe	OMe	5-OAc	OAc	OAc	OAc
6·00	6.05	**6·09**	6.12	6.15	—	—	—	—	—	—
6·04	**6·04**	6.07	6.12	—	—	—	—	—	—	—
6·05	6.10	6.11	6.13	6.31	—	—	—	7.68	7.78	—
6.03	6.08	6.12	6.34	—	—	—	7.47	**7·64**	7.75	—
6.08	**6·08**	6.37	—	—	—	—	**7·54**	7.65	7.65	7.86
6·00	**6·00**	6.09	—	—	—	—	—	—	—	—
5·97	6.06	6.06	6.06	6.06	6.06	6.23	—	—	—	—
6.09	6.09	6.35	—	—	—	—	7.53	7.65	768	7.76
5·90	**5·90**	**6·10**	—	—	—	—	—	—	—	—
6·05	6.05	6.05	605	6.05	6.20	—	—	—	—	—
6·05	6.05	**6·05**	6.05	6.12	6.28	—	**7·48**	—	—	—
5.99	**6·01**	6.05	6.12	—	—	—	—	—	—	—
6.07	607	6.07	6.18	—	—	—	**7·54**	7.63	**7·68**	—
5.93	5.97	5.97	6.02	6.08	—	—	—	—	—	—
6.07	6.07	6.10	6.10	6.13	—	—	—	**7·65**	7.65	—
602	**6·13**	6.13	—	—	—	—	—	—	—	—
6.09	6.12	6.35	—	—	—	—	**7·55**	7.70	7.80	—
6.10	6.17	6.17	—	—	—	—	—	—	—	—
6.04	6.10	**6·12**	629	—	—	—	—	7.66	**7·78**	—
6·13	613	6.39	—	—	—	—	**7·55**	**7·66**	7.78	—
6.02	6.12	6.12	—	—	—	—	—	—	—	—

into groups with identical oxygenation patterns, all were exhaustively methylated with dimethyl sulphate and K_2CO_3. Counting the number of the methoxyl groups of the derivatives by NMR spectroscopy revealed that five compounds were heptaoxygenated, while three were hexaoxygenated and one was pentaoxygenated.

Heptaoxygenated Flavones

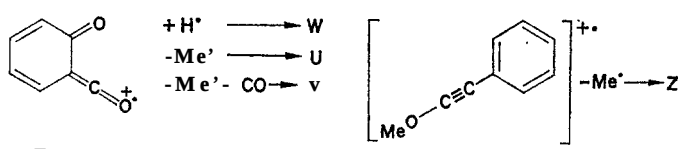
Two of the heptaoxygenated flavones yielded an identical methyl ether. A hydroxyl-methoxyl count revealed that one of the parent compounds, for which we propose the name apulein, is a dihydroxypentamethoxyflavone (Table 1, Compound Ia), and the other one is a trihydroxytetramethoxyflavone (Table 1, Compound Ib). The hydroxylproton signals in the NMR spectra of apulein (2.00 and 4·55τ) and of the trihydroxyflavone (-2.28, 2·13 and 4·67τ) indicated not only that a pair of OH groups may be situated in identical environments in both compounds, but also that the additional hydroxyl in the latter compound must be strongly chelated. The structure of 5-*O*-demethylapulein had thus to be considered for this compound. Aq. HCI promotes selective demethylation of 5-methoxyl groups in flavones[4] and the use of this reagent to convert apulein to the trihydroxy derivative confirmed their structural relationship.

The two remaining hydroxyls must be located in different environments, the one represented by a proton signal at about 2τ (Table I, Compounds Ia and b) being involved in a weak intramolecular hydrogen bond. Since a hydroxyl at position 3 gives rise to a proton signal at about 0·5τ,[5] the OH group in question was placed at C-2′, where it would be

TABLE 2. MASS SPECTRA OF *Apuleia* CONSTITUENTS AND OF THEIR METHYL ETHERS: PEAKS DUE TO CLEAVAGE OF THE HETEROCYCLE

Ion	*T*		*U*		*V*		*W*		*X*		*Y*		Z	
Compound	**m/e**	**%**	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
Ia	210	1	19.5	13	167	51	211	7	167		194	6	179	21
Ib	196	1	181	15	153	15	197	4	167	31	194	14	179	32
If	210	1	195	6	167	13	211	7	195		222	1	207	1
Va	182	3	167	5	139	3	183	13	167		194	9	179	8
Vb	210	2	195	8	167	12	211	10	195		222	1	207	3
Vd	182	5	167	17	139	1	183	5	331	1	358	16	343	5
VIa	182	5	167	2	139	2	183	5	181	6	208	2	193	3
VIe	196	2	181	7	153	7	197	4	181		208	1	193	3
VIIa	166	8	151	2	123	7	167	14	167		194	2	179	3
VIIIa	166	17	151	7	123	10	167	26	167		194	7	179	12
VIIIb	180	3	165	5	137	13	181	25	167	13	194	7	179	18
VIIIc	180	2	165	2	137	3	181	12	195	1	222	1	207	1
X	166	2	151	7	123	4	167	13	151		164	1	163	2

Key to Table-2.

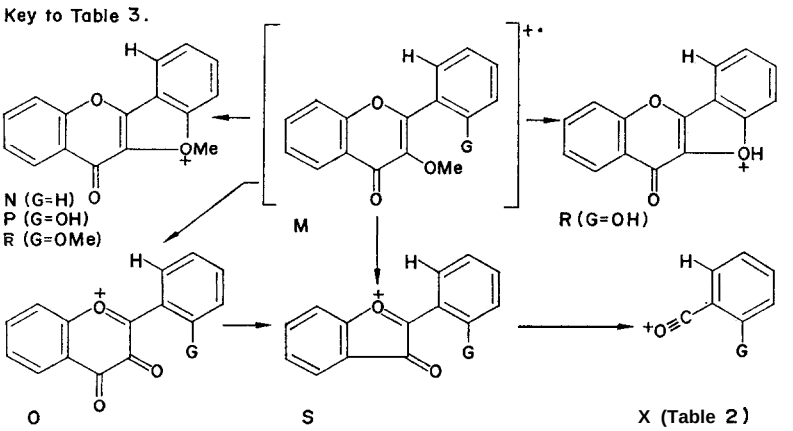


T Y

[4] F. SONDHEIMER and A. MEISELS, *Tetrahedron 9,143* (1960).
[5] T. J. BATTERHAM and R. J. HIGHET, *Australian J. Chem. 17,428 (1964).*

TABLE 3. MASS SPECTRA OF *Apuleia* CONSTITUENTS AND OF THEIR METHYL ETHERS: PEAKS DUE TO CLEAVAGE OF THE SUBSTITUENTS ON C-2' AND 3

Ion	M		N(M-1)		O(M-15)		P(M-17)		Q(M-30)		R(M-31)		S(M-43)	
Compound	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%	*m/e*	%
Ia	404	98	403	31	389	100	387	56	374	16	373	59	361	5
Ib	390	100	389	21	375	34	373	25	360	25	359	49	347	6
If	432	46	431	9	417	100			402	16	401	65	389	8
Va	376	100	375	23	361	14	359	36	346	3	345	16	333	9
Vb	432	42	431	13	417	100			402	13	401	58	389	2
Vd	540	100	539	22	525	2	523	2	510	1	509	3	497	7
VIa	390	100	389	33	375	33	373	6	360	18	359	15	347	10
VIe	404	100	403	43	389	71	387	10	374	8	373	10	361	4
VIIa	360	100	359	25	345	10	343	52	330	7	329	32	317	20
VIIIa	360	100	359	27	345	7	343	58	330	21	329	88	317	33
VIIIb	374	82	373	40	359	7	357	87	344	24	343	100	331	12
VIIIc	402	53	401	15	387	54			372	24	371	100	359	2
X	344	100	343	79	329	9	327	7	314	3	313	5	301	45



subjected to the polarizing influence of the heterocyclic oxygen. This seemed reasonable, if considered in conjunction with evidence pointing to the presence of the other hydroxyl at the *para* position: The UV spectrum of apulein is not altered upon addition of $AlCl_3$, H_3BO_3 or NaOAc,[6] but revealed that decomposition of the substance takes place upon addition of NaOH.

A preliminary indication of the localization of the four methoxy groups in the partial structures of apulein (IIa) and 5-*O*-demethylapulein (IIb) was obtained by mass spectrometry. The well known fragmentation mode, through compensated displacement of electrons in the heterocycle,[7,8] led to ions (Table 2, Compounds Ia and Ib) whose mass revealed the presence of two of these methoxyls in ring A, while the third one must be located either on the heterocycle or, together with the fourth one, on ring B. These assignments, however,

[6] L. JURD, in *The Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN), p. 107, Pergamon Press, Oxford (1962).
[7] C. S. BARNES and J. L. OCCOLOWITZ, *Australian J.* Chem. 17,975 (1964).
[8] A. PELTER, P. STAINTON and M. BARBER, *J. Heterocyclic Chem. 2,262 (1965).*

are somewhat ambiguous. Not only are the pertinent ions of low relative abundance, but, as Table 2 also shows, fragments of identical mass may be formulated as arising either from ring A (ions V) or ring B (ions X). Confirmation was secured through the more reliable information contained in Table 3. Loss of 43 mass units from the molecular ion, as featured in the reactions M→ 0 → S and M → S, is typical of 3-methoxyflavones.[9] Since at this stage the presence of a hydroxyl at position 2' had already been ascertained, the highly favoured losses of 17 (M → P) and of 31 mass units (M → R) were to be expected[10] and constitute additional evidence for the presence of a methoxyl group at C-3.

Mass spectral analysis thus authorized the expansion of the partial formula IIa and b respectively to IIIa and b. The proton signals in the aromatic region of the NMR spectra of all apulein derivatives (Table 1, Compounds Ia–e) were represented by singlets, indicating the absence of ***ortho*** or *meta* related aromatic hydrogens. This fact assigned the methoxyl of ring B to position 4'. The NMR signals at 2·7 and 3·4τ had, consequently. to be correlated with the protons at C-6' and C-3'. The resonance frequency of the remaining aromatic proton, however, was compatible with its presence either at C-6 or at C-8, and thus was not immediately helpful to decide unequivocally between two structural alternatives.

Definite proposals concerning the structures of apulein and of 5-0-demethylapulein could be advanced only after degradation experiments. Alkaline hydrolysis of apulein led to a mixture of 6′-hydroxy-2,2′,3′,4′-tetramethoxyacetophenone and of 6-hydroxy-2,3,4-trimethoxybenzoic acid. These fragments must correspond to ring A, since under the conditions ring B must have been destroyed. The substitution pattern of ring B could be defined after alkaline degradation of di-0-ethylapulein which led, in addition to the previous fragments, to 4-methoxy-2,5-diethoxybenzoic acid. Di-0-methylapulein, when submitted to the reaction, yielded, 2,4,5-trimethoxybenzoic acid. All these degradation products were characterized by physical and spectrometric means and by direct comparison with authentic samples. Clearly, they not only defined the localization of all oxygen functions, but also corroborated the assumption that the compounds are flavones. Accordingly, apulein must be 2′,5′-dihydroxy-3,4′,5,6,7-pentamethoxyflavone (Ia) and 5-0-demethylapulein must be 2′,5,5′-trihydroxy-3,4′,6,7-tetramethoxyflavone (Ib).

Once the structures of the apuleins had been established, it became necessary to re-interpret an experimental fact which did not fit the 5,6,7-oxygenation pattern for the apuleins. Nitric acid is known to effect oxidative demethylations leading to *para*-quinones[11] and treatment of apulein and of 5-0-demethylapulein with this reagent led to a single red quinone. This was reduced to a quinol which was acetylated without purification. NMR spectrometry revealed the product to be the trimethoxytetraacetoxyflavone of structure Ie, since a signal at relatively low field places one of the four acetyl groups at C-5 of the flavone skeleton.[12] Treatment of apulein and of 5-*O*-demethylapulein with nitric acid thus afforded the quinone IV and it must be concluded that nitric acid produces not only *para*, but also ortho-quinones. A reaction of this type, involving chromic oxide-acetic acid as the reagent, has been reported .[13]

The oxygenation pattern of apuleisin, a third heptaoxygenated flavone of *Apuleia leiocarpa,* differs from the pattern of the apuleins only with respect to ring B. Indeed, the fully 0-methylated derivative of this tetrahydroxytrimethoxyflavone yielded, upon treat-

[9] J. H. BOWIE and D. W. CAMERON, *Australian J.Chem.***19,** 1627 (1966).
[10] A. PELTER and P. STANTON, *J. Chem. Soc. (C)* 1933 (1967).
[11] M. KRISHNAMURTI, T. R. SESHADRI and P. R. SHANKARAN, *Tetrahedron* **22,** 941 (1966).
[12] J. MASSICOT, J.-P. MARTHE and S. HEITZ, *Bull. Soc. Chim. 2712 (1963).*
[13] D. KUMARI, S. K. MUKERJEE and T. R. SESHADRI, *Tetrahedron Letters 3767 (1966).*

ment with alkali, 2,3,4-trimethoxybenzoic acid, besides the 6′-hydroxy-2,2′,3′,4′-tetramethoxyacetophenone which had also been obtained upon degradation of the apuleins. The *ortho* relationship of the two ring B protons which was thus indicated, could also be appreciated through examination of the NMR spectrum of apuleisin (Table 1, Compound Va) which shows two *ortho* split doublets at 2·76 and 3·26 τ.

As in 5-*O*-demethylapulein, two of the free hydroxyls in apuleisin must occupy positions 5 and 2′, since their protons also gave rise to NMR signals respectively at −2·24 and 2·02 τ. Both these hydroxyls form part of ortho-dihydroxy systems. This was ascertained by heating apuleisin with dichlorodiphenylmethane. As indicated by mass spectrometry (Tables 2, and 3, Compound Vd), a diphenylmethylenedioxydihydroxy trimethoxyflavone resulted. The two hydroxyls of this derivative form an ortho-quinol function involving the hydroxyl at C-5, since the compound is labile in alkali and originates a PMR signal at −2·53 τ (Table 1, Compound Vd). On this evidence, the structure 2′,3′,5,6-tetrahydroxy-3,4′,7-trimethoxyflavone (Va) is proposed for apuleisin.

Apuleitrin and apuleirin, the fourth and fifth heptaoxygenated flavones of ***Apuleia Zeiocarpa,*** again yielded an identical methyl ether. Apuleitrin differs structurally from apuleisin (Va) only with respect to ring B. Indeed, while its mass spectrum (Tables 2 and 3, Compound VIa) reproduces fully all peaks originating through fragmentation of ring A of apuleisin, alkaline degradation of tri-O-methylapuleitrin afforded 3,4,5-trimethoxybenzoic acid. The structure of this methyl ether must consequently be represented by VIb. Confirmation was obtained by direct comparison with 3,3′,4′,5,5′,6,7-heptamethoxyflavone prepared by methylation of 3′,5,5′-trihydroxy-3,4′,6,7-tetramethoxyflavone (VIc) kindly supplied by Prof. P. R. Jefferies.[14]

The mass spectral indication of the substitution of ring A in apuleitrin was confirmed by NMR and UV spectrometry. The existence of a chelated hydroxyl (-2.43 τ, Table 1, Compound VIa) and of an ortho-dihydroxy-system (positive shift in u.v. spectrum with borate) was easily demonstrated. At this point only the relative site of one hydroxyl and two methoxyls at positions 3′, 4′ and 5′ remained to be established. The protons at C-2′ and 6′ of apuleitrin gave rise to a single NMR band at 2·64 τ (Table 1, Compound VIa). In spite of this fact, ring B must be asymmetrically substituted, because the corresponding signals in the spectrum of tri-O-acetylapuleitrin were characterized by two distinct chemical shift values (Table 1, Compound VId). Only 3′,5,6-trihydroxy-3,4′,5′,7-tetramethoxyflavone (VIa) can thus represent the structure of apuleitrin.

Apuleirin is a dihydroxypentamethoxy derivative (Table 1, Compound VIe). Since the structure of its methyl ether VIb had already been determined, only the relative positions of the substituents remained to be established. NMR (Table 1, Compound VIe) and UV data (no shift with $AlCl_3$) showed the absence of hydroxyls from both the 3- and 5-positions. The NMR spectrum contains a two-proton singlet at 2·55 τ which can only be attributed to the hydrogens at C-2′ and 6′. The magnetic equivalence of these protons does not persist in apuleirin acetate, since they are represented by two *meta*-split doublets in the spectrum of this derivative (Table 1, Compound VIf). Asymmetric substitution of ring B requires the presence of a hydroxyl at C-3′. The only alternative asymmetric substitution pattern, namely a 3′,4′-dihydroxy-5′-methoxy grouping, could be ruled out, because apuleirin is stable in alkali and no alteration of its *w* spectrum was observed upon addition of H_3BO_3. Absence of a paramagnetic shift of the H-8 NMR signal upon acetylation, and a bathochromic shift of the UV band II upon addition of NaOAc,[6] can only be rationalized if

[14] P. R. JEFFERIES, J. R. KNOX and E. J. MIDDLETON, *Australian J. Chem.* 15,532 *(1962).*

the remaining OH group is at C-6. The mass spectral data (Tables 2 and 3) are in accord with the structure of 3′,6-dihydroxy-3,4′,5,5′,7-pentamethoxyflavone (VIe) which was consequently assigned to apuleirin.

Hexaoxygenated Flavones

The distribution of hydroxyls and methoxyls on the skeleton of apuleidin, one of the hexaoxygenated flavones, was determined by mass spectrometry (Table 3, Compound VIIa). The masses of ions of types *T*, *U* and *V* (Table 2, Compound VIIa) indicated the existence of a hydroxy-methoxylated A-ring, while the masses of the ions Y and *Z* were compatible with a dihydroxy-methoxylated B-ring. The UV spectrum revealed the vicinal relationship of the two hydroxyls in ring B (spectral shifts with H_3BO_3 and decomposition in NaOH). Consequently, when the characteristic bands at -2.46 and 1·95 τ were found in the NMR spectrum of apuleidin (Table 1, Compound VIIa), the location of the hydroxyls at positions 5 and 2′,3′ had to be considered. The same spectrum indicated also the presence of four aromatic protons in *meta* and *ortho* related pairs. The chemical shifts of the corresponding signals suggested their situation respectively on ring A and ring B. All these data lead to structure 2′,3′,5-trihydroxy-3,4′,7-trimethoxyflavone (VIIa) for apuleidin.

The fully 0-methylated derivatives of the two other hexaoxygenated flavones isolated from *Apuleia leiocarpa* were identical. Mass spectra characterized one of the parent compounds (Table 3, Compound VIIIb) as a dihydroxy tetramethoxyflavone and the other one (Table 3, Compound VIIIa) as a trihydroxytrimethoxyflavone. The NMR spectra indicated, through a proton signal at -2.35 τ, the presence of a 5-OH group only in the trimethoxyflavone (Table 1, Compound VIIIa). This suggested again a 5-OMe/5-OH relationship and, indeed, the tetramethoxyflavone was easily converted into the trimethoxyflavone upon heating with aqueous HCl.

UV, NMR (Table 1, Compound VIIIa) and mass spectral (Tables 2 and 3, Compound VIIIa) measurements, as well as degradation by alkali (see Experimental), indicated that the 5-hydroxy-compound was 2′,5,5′-trihydroxy-3,4′,7-trimethoxyflavone (VIIIa) and the 5-methoxy-compound 2′,5′-dihydroxy-3,4′,5,7-tetramethoxyflavone (VIIIb). VIIIa is the structure of oxyayanin-A, a constituent of *Distemonanthus benthamianus*.[15]

Jain *et al.*[16] synthesized 2′,5,5′-trihydroxy-3,4′,7-trimethoxyflavone (VIIIa) and found it to be different, by IR spectroscopy, from a sample of oxyayanin-A supplied by Prof. F. E. King, although they recorded close agreement in colour reactions, UV spectra and m.p. This, in addition to the fact that the reported[15] procedure for the isolation of oxyayanin-A from *Distemonanthus benthamianus* included alkaline treatment which destroys VIIIa, forced the Indian workers to consider the structural proposal VIIIa advanced for the natural compound as erroneous,[16,17] and stimulated Dreyer and Bertelli to suggest that a 2′,6′-disubstituted B-ring might be present in oxyayanin-A.[18] Such a hypothesis would, of course, never have been formulated, had the authors been able to examine the NMR spectrum. This shows (Table 1, Compound VIIIa) two doublets whose chemical shifts and coupling constant allow correlation only with protons at C-6 and C-8, and two singlets revealing a pair of *para* related protons. These can only be placed on ring B, which must consequently be substituted at positions 2′,4′ and 5'.

[15] F. E. KING, T. J. KING and P. J. STOKES, *J. Chem. Soc.* 4587 (1954).
[16] A. C. JAIN, S. K. MATHUR and T. R. SESHADRI, *Indian J. Chem. 4,365 (1966).*
[17] *S. C.* DATTA, V. V. *S.* MURTI and T. R. SESHADRI, *Zndian J. Chem. 7,110* **(1969).**
[18] D. L. DREYER and D. J. BERTELLI, *Tetrahedron* **23,** 4607 **(1967).**

We were able to identify the 5-hydroxyflavone of ***Apuleia leiocarpa*** with oxyayanin-A by direct comparison (IR and UV spectra, TLC and mixed m.p.) with an authentic sample from Dr. T. J. King. Consequently, when Prof. T. R. Seshadri kindly informed us that he found our oxyayanin-A ex ***A, leiocarpa*** identical with his synthetic VIIIa, the structural proposal originally advanced for oxyayanin-A was established as correct, and the remaining question concerned only the nature of the sample which had been sent from Nottingham to Delhi.

This problem was solved when it was found that oxyayanin-A, crystallized from ethyl acetate-light petroleum, ethyl acetate-benzene or methanol, gave an i.r. spectrum (in KBr) identical with that published by Seshadri ***et al.***[16] for synthetic VIIIa. When oxyayanin-A, however, was dissolved in chloroform and the solvent removed by evaporation, the residue, although indistinguishable from oxyayanin-A by UV all other methods, gave rise to an IR spectrum (in KBr) identical to that published by Seshadri ***et al.***[16] for the natural oxyayanin-A received from England.

Distemonanthus benthamianus Baill. is an African timber closely akin to ***Apuleia leiocarpa.***[19] Interestingly, both species belong to monotypic genera. The finding of oxyayanin-A in ***A. leiocarpa*** stimulated the search for other constituents of ***D. benthamianus*** in the Brazilian species. Indeed, the presence in the corresponding extract of still another hexaoxygenated flavone, namely oxyayanin-B (IX),[15] could be demonstrated by TLC.

Pentaoxygenated Flavone

The sole pentaoxygenated flavone isolated from ***A. leiocarpa*** was, at this stage, identified easily through spectral measurements (cf. Tables l-3, Compound X; Experimental) with yet another constituent of ***D. benthamianus,*** namely ayanin (X).[20]

Mass Spectra

Table 4 summarizes some of the mass spectral data contained in Table 2. Clearly, the substitution at C-2' and 6' of 3-methoxyflavones can be recognized upon examination of the relative abundances of their M-l, M-17 and M-31 ions. Absence of substitution confers high stability to the molecular ion, favouring only the expulsion of a hydrogen atom. Substitution by hydroxyl favours the expulsion of OH and OMe radicals, while substitution by methoxyl favours expulsion only of OMe.

TABLE 4. ABUNDANCE OF IONS *M, N, P* AND *R* IN THE MASS SPECTRA OF THE 2'-G-SUBSTITUTED 3-METHOXYFLAVONES LISTED IN TABLE 2

Ion	G = H	G = OH	G = OMe
M	100	*82-100*	*42-53*
M-l	33–79	21-40	9-15
M-17	6-10	25-87	
M-31	5-15	16-100	58-100

[19] A. ENGLER and K. PRANTL, *Die natürlichen Pflanzen-familien*, Vol. 3, Part 3, p. 156, Engelmann, Leipzig (1894).
[20] F. E. KING, T. J. KING and K. SELLARS, *J. Chem. Soc. 92* (1952).

Leiocarpin

The aromatic constituent of ***A. leiocarpa*** bark was classified as a pterocarpan by means of its very typical NMR[21–23] and UV[24] spectra. The mass spectrum suggested, through its molecular ion, substitution by a methylenedioxy- and a 2,2-dimethylchromene unit. The prominent M-15 peak was attributed to the benzopyrilium ion, formed by loss of a methyl radical from the chromene portion of the molecule. Direct assignment of the substituents to rings A or B of the pterocarpan skeleton through mass spectrometry was not feasable for, as shown previously,[8,25] the major fragments can be reasonably formulated as arising from either ring (e.g. XI, XII). As expected, however, the interpretation of the mass spectrum of the isoflavan, derived from leiocarpin by catalytic hydrogenation, was straightforward. The retro-Diels Alder fragmentation mode yielded the ions XIII and XIV in high relative abundance. The 2,2-dimethylchromane-group can thus be located only on ring A, while the methylenedioxy-group must occupy positions of ring B.

The aromatic region of the NMR spectrum of the isoflavan showed four bands, each representing one proton : two doublets centered at 3·16 and 3·70 τ (J 8·6 Hz) and two singlets at 3·31 and 3·49 τ. Clearly, the chemical shift of the low field signal reveals the existence of a proton which keeps neither an ***ortho-*** nor ***apara*** relationship to an oxy-group,[26] a condition which can be met only by a H at C-5 of a C-7-oxygenated A-ring. Since the proton signal under scrutiny is characterized by an ortho-coupling constant, a second H must occur at C-6. The ring B protons gave rise to two singlets and must be *para* related. Structure XV had consequently to be assigned to the tetrahydrogenated derivative of leiocarpin.

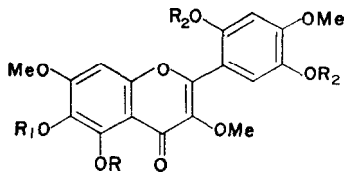
The 3S-configuration for the isoflavan XV was deduced upon inspection of its ORD curve. This showed a negative Cotton effect in the 260-300 nm region, in analogy with the curves of known 3S-isoflavans[27] which do not bear an additional substituent at C-6.[25]

It was now possible to formulate structure and configuration of leiocarpin (XVI) on the reasonable assumption that the heterocyclic rings are ***cis*** fused.[28] Confirmation of the structure was provided by all features of the NMR spectrum. Observed (3·31, 3·58 τ) and expected (3·40, 3·66 τ)[25] chemical shift values for the singlets due to the C-7 and C-10 protons are in agreement. Confirmation of the absolute configuration was obtained by inspection of the ORD curve. This followed, between 3.50 and 220 nm, precisely the course expected for 6aS,11aS-pterocarpans.[25,29]

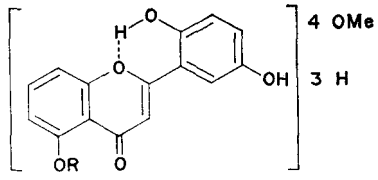
DISCUSSION

Methylation of the 4′-hydroxyl in preference to the 3’-hydroxyl is relatively rare in natural flavonoids.[30] Two additional conspicuous examples are eupatin (XVIIa) and eupatoretin (XVIIb), not only on account of their close structural relationship with oxyayanin-B (IX), but also because they show moderate cytotoxicity against human carcinoma of the nasopharynx.[31]

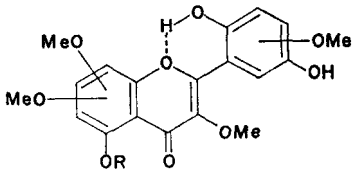
[21] D. R. PERRIN, *Tetrahedron Letters 29 (1964).*
[22] K. G. PACHLER and W. G. E. UNDERWOOD, *Tetrahedron* **23,** 1817 *(1967).*
[23] K. FUKUI, M. NAKAYAMA and T. HARANO, *Bull. Chem. Soc. Japan 42,233 (1969).*
[24] B. L. VAN DUUREN, *J. Org. Chem.* **26,** 5013 *(1961).*
[25] A. PELTER and P. I. AMENECHI, *J. Chem. Soc. (C) 887 (1969).*
[26] J. A. BALLANTINE and C. T. PILLINGER, *Tetrahedron* **23,** 1691 *(1967).*
[27] K. KUROSAWA, W. D. OLLIS, R. T. REDMAN, I. O. SUTHERLAND, O. R. GOTTLIEB and H. MAGALHÃES ALVES, *Chem. Commun. 1265* (1968).
[28] H. SUGINOME, *Bull. Chem. Soc. Japan 39,409* **(1966).**
[29] H. SUGINOME and T. IWADARE, *Experientia* **18,163 (1962).**
[30] C. A. HENRICK and P. R. JEFFERIES, *Australian J. Chem. 17,934 (1964).*
[31] S. M. KUPCHAN, C. W. SIGEL, J. R. KNOX and M. S. UDAYAMURTHY, *J. Org. Chem. 34, 1460 (1969).*



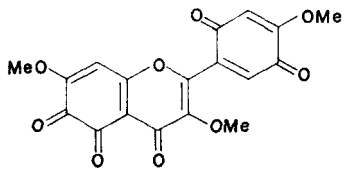
	R	R_1	R_2	
Ia	Me	Me	H	apulein
b	H	Me	H	**5-*O*-demethylapulein**
c	Me	Me	AC	
d	Ac	Me	Ac	
e	A c	A c	Ac	
f	Me	Me	Me	
g	Me	Me	Et	
h	H	H	H	



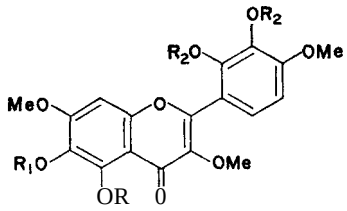
IIa R = M e
b **R = H**



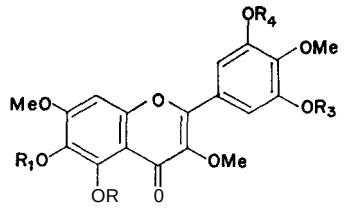
IIIa R - M e
b R - H



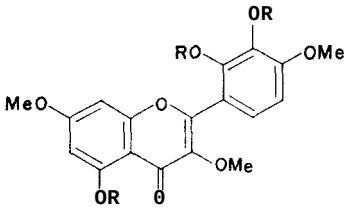
IV



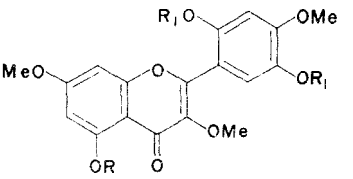
	R	R_1	R_2	
Va	H	H	H	apuleisin
b	Me	Me	Me	
c	Ac	A c	A c	
d	**H**	H	**CPh2**	
e	H	Me	Me	
f	Ac	Me	Me	
g	Et	Et	Et	



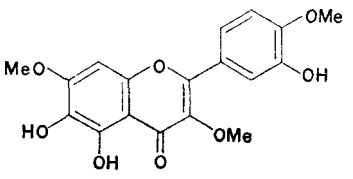
	R_1	R_2	R_3	R_4	R_5	
VIa	H	H	Me	H	Me	apuleitrin
b	Me	Me	Me	Me	Me	
c	H	Me	Me	H	H	
d	Ac	A c	Me	Ac	Me	
e	Me	Me	H	H	Me	**apuleirin**
f	Me	Me	Ac	A c	Me	



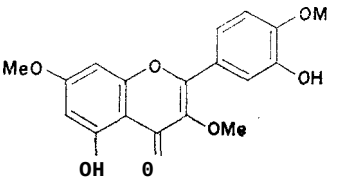
	R	
VIIa	H	apuleidin
b	Ac	



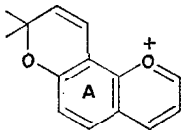
	R	R_1	
VIIIa	H	H	oxyayanin-A
b	Me	H	5-*O*-methyloxyayanin-A
c	Me	Me	
d	Me	Ac	
e	Ac	Ac	



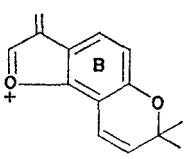
Ix oxyayanin-B



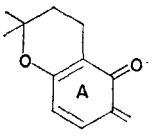
X ayanin



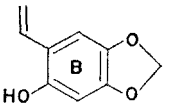
XI m/e 213



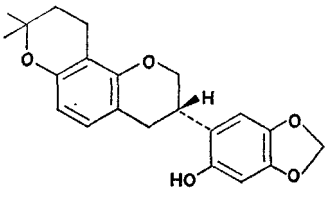
XII *m/e* 213



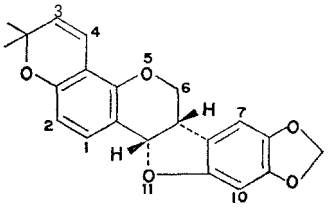
XIII *m/e 190* (1%) 191 (98%)



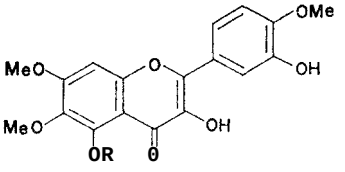
XIV m/e 164 (100%)



XV



XVI leiocarpin



XVIIa	R = H	eupatin
b	R = Me	eupatoretin

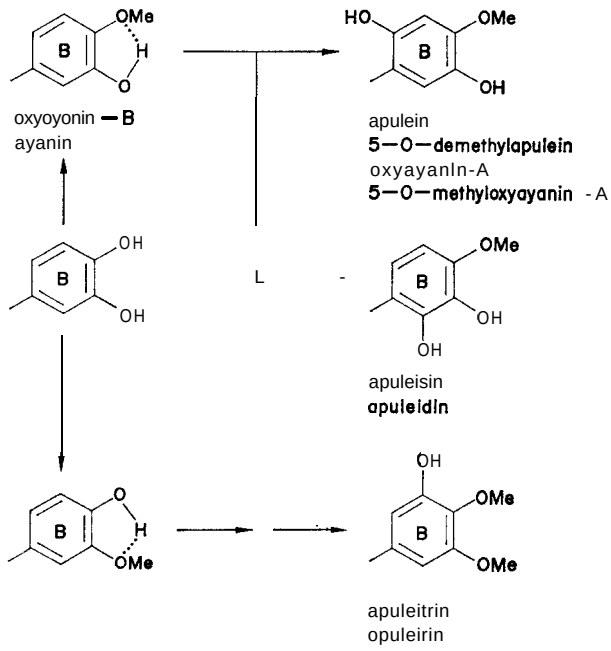


CHART 1. POSTULATED DERIVATION OF B-RING OXYGENATION IN FLAVONES ISOLATED FROM *A. leiocarpa.*

The postulated derivation of trioxygenated B-rings of ***Apuleia*** constituents from dioxygenated precursors is presented in Chart 2. It seems obvious that the hydroxyl, and not the methoxyl, controls the orientation of electrophyllic attack by ^{+}OH to its *para* or *ortho*-positions. Effects of this nature were attributed to the ability of the hydroxyl groups to establish a hydrogen bond with the neighbouring methoxy group.[32] As a result of such intramolecular hydrogen bonding, mesomeric and inductive electron release toward *ortho* or *para* positions would be expected to be enhanced for the hydroxyl, and repressed for the methoxyl. This may explain why the usual 4′-hydroxy-3′-methoxy substituion in natural diarylpropanoids leads to the usual 3′,4′,5′-trioxygenated derivatives, while, at least in ***Distemonanthus*** and ***Apuleia*** species, the unusual 3′-hydroxy-4′-methoxy substitution leads to the unusual 2′,4′,5′- and 2′,3′,4′-trioxygenation patterns.

That the 3′,4′,5′-oxygenation pattern is indeed usual among flavonoids was again brought out in the present work with respect to apuleitrin (VIa) and apuleirin (VIe). Identically substituted derivatives have been located in such unrelated species as *Eremophila fraseri* F. Muell. (Myoporaceae)[14] and *Murraya paniculata* (L.) Jack, (Rutaceae).[33] In contradistinction, while 2′-hydroxylated B-rings are reasonably widespread among the isoflavonoids of the Leguminosae,[34] and a proposal has been advanced which rationalizes this fact mechanistically,[35] oxyayanin-A was until now the only 2′-hydroxyflavanoid

[32] M. BARROETA, G. CHUCHANI and J. ZABICKY, *J. Org. Chem.* **31**, 2330 (1966).
[33] D. L. DREYER, *J. Org. Chem.* **33**, 3574 (1969).
[34] H. GRISEBACH and W. D. OLLIS, *Experientia* **11**, 4 (1961).
[35] W. D. OLLIS, in *Recent Advances in Phytochemistry* (edited by T. J. MABRY, R. E. ALSTON and V. C. RUNECKLES) Vol. 1, p. 348, Appleton-Century-Crofts, New York (1968).

reported in the family. Nevertheless, recent papers[17,36,37] mention 27 additional representatives of this class from members of the Acanthaceae, Datiscaceae, Labiatae, Meliaceae, Moraceae and Rutaceae. Their biosynthesis, however, does not necessarily follow the course which is suggested for the *Apuleia* constituents, and at least some of these compounds may be derived by some departure from the usual acetate-shikimate pathway.[38]

Finally, it has been reported that ayan wood *(Distemonanthus benthamianus)* may cause dermatitis due to the presence of oxyayanin-A and oxyayanin-B.[39] Clearly, this is another indication designed to stimulate an investigation into the physiological activity of the constituents of garapa wood *(Apuleia leiocarpa).*

EXPERIMENTAL

NMR spectra were determined in $CDCl_3$ using TMS as an internal standard. M.ps were determined using a Kofler hot stage microscope and are uncorrected. Separations by column chromatography were carried out usine Merck Kieselgel 0·05–0·20 mm. Merck's Kieselgel G was used for TLC. During isolation processes the appropriate combination of fractions was determined by examination of their i.r. spectra and TLC behaviour. TLC plates were examined under UV illumination and after exposure to iodine vapour. The majority of NMR and mass spectral figures are given in Tables 1-3.

Isolation of Apuleia Constituents

Benzene extraction of the heartwood of A. leiocarpa. *Isolation of ten flavones and β-sitosterol.* The powdered heartwood (5·0 kg) was continuously extracted with hot benzene. Upon partial evaporation of the solvent separated an oil which solidified by standing (Hi). Further concentration of the benzene solution produced a crystalline precipitate which was separated into an acetone insoluble (H_2) and an acetone soluble portion. The acetone soluble portion was chromatographed on silica giving fractions H_3–H_6, in this order, by elution with benzene-AcOEt(l:l). Two more successive crops of crystals (H_7 and H_8) of the original benzene solution were collected. The remaining benzene solution was divided into two parts, H_9 and H_{10}.

Hi was washed with a small quantity of benzene, and freed from an insoluble portion by treatment with $CHCl_3$. The $CHCl_3$-solution was evaporated. The residue, purified by passage of its methanol solution through a column of Sephadex LH-20 gelified with methanol, and recrystallizations from the same solvent, afforded apuleisin (Va, 530 mg).

H_2 was recrystallized from acetone yielding apulein (Ia, 1·5 g). H_3 was recrystallized from benzene-AcOEt yielding oxyayanin-A (VIIIa, 160 mg). H_4 was recrystallized from benzene yielding 5-*O*-demethylapulein (Ib, 180 mg). H_5 was recrystallized from acetone yielding apulein (Ia, 900 mg). H_6 was recrystallized from acetone yielding 5-*O*-methyloxyayanin-A (VIIIb, 140 mg). H_7 was recrystallized from acetone, purified by chromatography on silica, elution with benzene-AcOEt(1 :1), and recrystallization from methanol, yielding ayanin (X, 9 mg). H_8 was recrystallized from ethanol yielding apuleisin (Va, 40 mg). H_9 was evaporated. The ethanol soluble part of the residue was chromatographed on a polyamide column. Benzene–MeOH 12:1 eluted a product in which the presence of oxyayanin-B (IX) was detected by TLC (silica $CHCl_3$).

H_{10} was partially evaporated. An oil precipitated and was separated. Treatment of this oil with MeOH produced a crystalline mass which was shown by TLC to be composed of 5-*O*-demethylapulein (Ib), apuleisin (Va) and oxyayanin-A (VIIIa). The filtered MeOH solution was concentrated to a small volume and chromatographed through a column of Sephadex LH-20 gelified with MeOH. In order of elution were collected β-sitosterol (30 mg), a fraction which after recrystallization from AcOEt-light petroleum yielded apuleitrin (VIa, 30 mg), and a fraction which after recrystallization from MeOH yielded ayanin (X, 25 mg).

Further evaporation of benzene from the original H_{10}-solution afforded a second crop of oily precipitate which was again treated with MeOH. The crystals were collected and again shown by TLC to be composed of 5-*O*-demethylapulein (Ib), apuleisin (Va), and oxyayanin-A (VIIIa). The filtered MeOH solution was evaporated and the residue dissolved in $CHCl_3$. This solution was extracted several times with conc. aqueous borax. The aqueous layers were united, washed with $CHCl_3$, acidified with HCl and extracted with $CHCl_3$. The $CHCl_3$-solution was washed with water, dried, evaporated and the residue (1.9 g) chromatographed on silica (60 g). In order of elution with benzene-AcOEt were collected a fraction which

[36] P. C. PARTHASARATHY, P. V. RADHAKRISHNAN, S. S. RATHI and K. VENKATARAMAN, *Indian J. Chem. 7,* 101 (1969).
[37] T. R. GOVINDACHARI, B. R. PAI, M. SRINIVASAN and P. S. KALYANARAMAN, *Indian J. Chem. 7, 306 (1969).*
[38] D. L. DREYER, *J. Org. Chem.* **33,** 3577 (1968).
[39] J. W. W. MORGAN and J. THOMSON, *Brit. J. Ind. Med. 24,156 (1967).*

after recrystallization from CCl_4 yielded apuleidin (VIIa, 129 mg), a fraction which after recrystallization from acetone-light petroleum yielded apuleitrin (VIa, 109 mg), and a fraction which after recrystallization from benzene–MeOH yielded apuleirin (VIe, 22 mg). In the mother-liquor, which remained after the filtration of apuleitrin, the presence of oxyayanin-B (IX) was demonstrated by TLC.

Ethanol extraction of the heartwood of A. leiocarpa. ***Isolation of (+)-pinitol.*** The ground wood, after extraction with hot benzene, see above, was continuously extracted with EtOH. Partial evaporation of the solvent produced a crystalline deposit which through recrystallization from EtOH yielded (+)-pinitol (72 mg).

Benzene extraction of the sapwood of A. leiocarpa. The powdered sapwood (10·0 kg) was continuously extracted with hot benzene. Upon removal of the solvent a residue (100 g) remained. Column or TLC revealed the presenceof apulein (Ia), 5-*O*-demethylapulein (Ib), oxyayanin-A(VIIIa), 5-*O*-methyloxyayanin-A (VIIIb), apuleisin (Va), apuleitrin (VIa), ayanin (X) and β-sitosterol.

Benzene extraction of the bark of A. leiocarpa. ***Isolation of β-sitosterol and Ieiocarpin*** (XVI). The powdered bark (1.2 kg) was continuously extracted with hot benzene.' Evaporation of the solvent yielded a residue (16 g), part of which (3 g) was chromatographed on silica (90 g). Elution with benzene-$CHCl_3$ (9:1) afforded β-sitosterol (70 mg) and a fraction which after recrystallization from EtOH yielded leiocarpin (XVI, 130 mg).

Apulein and Derivatives

Apulein (Ia). Pale yellow rectangular plates, m.p. 211-213". λ_{max}^{MeOH} 250, 315, 335 infl. (ε 21,450, 12,400, ll,lOO), $\lambda_{max}^{MeOH+NaOH}$ 244, 287, $\lambda_{max}^{MeOH+NaOH+HCl}$ 238, 290, unchanged on addition of NaOAc, H_3BO_3 + NaOAc or $AlCl_3$; Gibbs test, λ_{max} 450, 690 nm. ν_{max}^{KBr} (cm-'): 3330, 1620, 1605, 1558, 1510.

5-*O*-*Demethylapulein* (Ib). Orange needles, m.p. 228–230°. λ_{max}^{EtOH} 260, 305, 350 (ε 40,100, 15,950, 20,650), $\lambda_{max}^{EtOH+NaOH}$ 270, 360, $\lambda_{max}^{EtOH+NaOH+HCl}$ 255,298, $\lambda_{max}^{EtOH+AlCl_3}$ 267, 314, 357, unchanged in presence of NaOAc, H_3BO_3 + NaOAc. ν_{max}^{KBr} (cm-'): 3458, 1658, 1631, 1600,1563, 1508, 1493.

Apulein diacetate (Zc). White needles from hexane-benzene, m.p. 158-160". λ_{max}^{EtOH} 230, 254, 308 nm (ε 24,650, 19,050, 21,450). ν_{max}^{KBr} (cm-'): 1768, 1640, 1613, 1565, 1517. M.S.: M^+ 488 (75%); *m/e* (%) 474 (17) 473 (58), 446 (23), 445 (25), 431 (50), 430 (23), 429 (94), 416 (13), 415 (69), 404 (33), 403 (100), 389 (8). 388 (30), 387 (31), 386 (8), 385 (38), 383 (12), 375 (6), 374 (12), 373 (54), 371 (15), 369 (5), 359 (7), 357 (11), 355 (11), 345 (8), 343 (14), 341 (5), 329 (13), 327 (5), 315 (5), 211 (12), 202 (6), 195 (21), 194 (8), 188 (7), 181 (8), 180 (5), 179 (22), 168 (6), 167 (65), 165 (6), 153 (12), 151 (10), 150 (8), 149 (8), 139 (10), 137 (8), 136 (6), 135 (5), 123 (6), 122 (8), 109 (7), 107 (6), 105 (5).

5-*O*-*Demethylapulein triacetate (Id).* White rectangular plates from EtOH, m.p. 198–200°. λ_{max}^{EtOH} 233, 254, 310 nm (ε 24,350, 17,250, 20,950). ν_{max}^{KBr} (cm-'): 1773, 1645, 1631, 1582,1515, 1493.

Apulein dimethyl ether (Zf). White rectangular plates from methanol (463 mg), m.p. 159–160°; λ_{max}^{EtOH} 245, 320 nm (ε 23,200, 16,200), no alteration with NaOH. ν_{max}^{KBr} (cm-'): 1637, 1613, 1570, 1515. M.S.: Tables 2, 3. Methylation of 5-*O*-demethylapulein also afforded apulein dimethyl ether.

Apulein diethyl ether (Ig). White needles from benzene, m.p. 129-131". λ_{max}^{EtOH} 247, 320 nm (ε 21,700, 16,250), ν_{max}^{KBr} (cm-'): 1628, 1605, 1566, 1514.

Apuleisin and Derivatives

Apuleisin (Va). Yellow needles, m.p. 193-195". λ_{max}^{MeOH} 237, 272, 328 (ε 25,600, 21,600, 19,750) $\lambda_{max}^{MeOH+NaOAc}$ 237, 272, 332, $\lambda_{max}^{MeOH+H_3BO_3+NaOAc}$ 240, 277, 335, $\lambda_{max}^{MeOH+AlCl_3}$ 242, 280,385, $\lambda_{max}^{MeOH+AlCl_3+HCl}$ 270, 282, 350, Gibbs test: λ_{max} 460,590 nm. ν_{max}^{KBr} (cm-'): 3430, 1667, 1623, 1595, 1568.

Apuleisin tetramethyl ether (Vb). White needles from MeOH, m.p. 186–188°. λ_{max}^{EtOH} 235, 255, 308 nm (ε 27,050, 21,200, 17,500), ν_{max}^{KBr} (cm-'): 1632, 1588, 1491.

Apuleisin tetraacetate (Vc). White crystals from EtOH, m.p. 204–206°. λ_{max}^{EtOH} 230, 250, 308 nm (ε 24,100, 18,600, 19,800). ν_{max}^{KBr} (cm^{-1}): 1783, 1631, 1585, 1504.

2′,3′-*O*-*Diphenylmethylapuleisin (Vd).* A mixture of apuleisin (35 mg) and dichlorodiphenylmethane (200 mg) was maintained at 140–150° for 20 min. After cooling to room temp., benzene was added. The solid which precipitated (11 mg) was filtered. Upon addition of light petroleum to the filtrate an additional quantity of solid material (6 mg) was obtained. The united precipitates were crystallized from EtOH, yielding yellowish rectangular plates, m.p. 232-234". λ_{max}^{EtOH} 240, 280, 330 (ε 29,250, 29,400, 27,750), $\lambda_{max}^{EtOH+NaOH}$ 223, 255, 360, $\lambda_{max}^{EtOH+NaOH+HCl}$ 215, 260, $\lambda_{max}^{EtOH+H_3BO_3+NaOAc}$ 285, 325, $\lambda_{max}^{EtOH+AlCl_3}$ 240, 295, 363, no alteration with NaOAc. ν_{max}^{KBr} (cm-'): 3450, 2600 broad, 1645, 1590, 1555, 1536, 1499. Mass spectra: M^+ 540 (100%); m/e (%) 539 (22), 497 (7), 464 (5), 463 (17), 358 (16), 357 (10), 343 (5), 340 (6), 301 (6), 167 (17), 166 (6), 165 (12), 151 (5), 149 (8), 109 (5), 105 (40).

2′,3′,6-*Tri-O-methylapuleisin* (*Ve*). By treatment of apuleisin with CH_2N_2 white crystals from benzene–hexane, m.p. 136–138°. λ_{max}^{MeOH} 234, 261, 310 (ε 22,300, 20,050, 14,750), $\lambda_{max}^{MeOH+NaOH}$ 276, 370, $\lambda_{max}^{MeOH+NaOH+HCl}$ 234,261, 312, $\lambda_{max}^{MeOH+AlCl_3}$ 272, 295, 338, $\lambda_{max}^{MeOH+AlCl_3+HCl}$ 272,295 infl., 330 nm (ε 17,650, 11,500, 13,100), ν_{max}^{KBr} (cm-'): 3300, 2500 broad, 1648, 1600, 1570, 1490.

5-*O-Acetyl*-2′,3′6-*tri-O-methylapuleisin* (*Vf*). White crystals from hexane-benzene, m.p. 204–206° (open capilary). λ_{max}^{EtOH} 233, 250 infl., ***306*** (ε ***18,550, 13,400, 13,600***). ν_{max}^{KBr} (cm-r): 1750, 1635, 1620, 1570, 1495.

Apuleisin ***tetraethyl ether*** (*Vg*). White crystals from light petroleum-benzene, m.p.110–1 12". λ_{max}^{EtOH} 235, 252, 306 nm (ε 20,050, 15,300, 13,400). ν_{max}^{KBr} (cm-'): 1639, 1605, 1493.

Apuleitrin, Apuleirin and Derivatives

Apuleitrin (*VIa*). Yellow rectangular plates, m.p. 175-177". λ_{max}^{MeOH} 238,282, 340 (ε 13,600, 16,050, 19,050), $\lambda_{max}^{MeOH+NaOH}$ 240, 300, 365, $\lambda_{max}^{MeOH+NaOH+HCl}$ 235, 275, 314, $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ 240, 288, 335, $\lambda_{max}^{MeOH+AlCl_3}$ 240, 256, 298, 374, $\lambda_{max}^{MeOH+AlCl_3+HCl}$ 240, 256, 298, 365 nm, no alteration with NaOAc. ν_{max}^{KBr} (cm-'): 3333, 1653, 1575, 1550, 1504.

Apuleitrin trimethyl ether (*VIb*). White rectangular plates from light petroleum–AcOEt, m.p. 152-154" (lit.'" 155–156°). λ_{max}^{EtOH} 237, 260, 325 nm (ε 17,550, 13,050, 21,100). ν_{max}^{KBr} (cm-r): 1639, 1600, 1577, 1504. Methylation of apuleirin with CH_2N_2 also afforded apuleitrin trimethyl ether.

Apuleitrin triacetate (*VId*). White rectangular plates from MeOH, m.p. 215-218". λ_{max}^{EtOH} 230, 245, 320, 337 nm (ε 18,850, 14,950, 14,550, 14,050). ν_{max}^{KBr} (cm-'): 1780, 1770, 1632, 1612, 1570, 1500.

Apuleirin (*VIe*). Pale yellow crystals, m.p. 218-220". λ_{max}^{MeOH} 236, 270, 330 (ε 11,550, 8750, 14,200), $\lambda_{max}^{MeOH+NaOH}$ 253, 293, 340, $\lambda_{max}^{MeOH+NaOH+HCl}$ 236, 267, 330, $\lambda_{max}^{MeOH+NaOAc}$ 230, 240, 295, 332, $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ 235, 270, 330, $\lambda_{max}^{MeOH+AlCl_3}$ 235, 270, 330, $\lambda_{max}^{MeOH+AlCl_3+HCl}$ 235, 270, 330 nm. ν_{max}^{KBr} (cm-'): 3425, 1620, 1600, 1563, 1515.

Apuleirin diacetate (*VIf*). White crystals from light petroleum-benzene, m.p. 174-175". ν_{max}^{KBr} 1761, 1629, 1605, 1497, 1453, 1493 cm-r.

Apuleidin and Derivatives

Apuleidin (*VIIa*). Pale yellow rectangular plates, m.p.154–156°. λ_{max}^{MeOH} 230, 260, 300, 333 (ε 10,650, 11,250, 5300, 6700), $\lambda_{max}^{MeOH+NaOH}$ 265, 360, $\lambda_{max}^{MeOH+NaOH+HCl}$ 255, 290, 320, $\lambda_{max}^{MeOH+NaOAc}$ 238, 255, 300, 335, $\lambda_{max}^{MeOH+NaOAc+H_3BO_3}$ 233, 260, 295, 343, $\lambda_{max}^{MeOH+AlCl_3}$ 268, 300, 360, 400, $\lambda_{max}^{MeOH+AlCl_3+HCl}$ 270, 300, 334, 38Snm. ν_{max}^{KBr} (cm-'): 3500, 1650, 1620, 1600, 1570, 1500.

Apuleidin triacetate (*VIIb*). White rectangular plates from EtOH, m.p. 198-200". λ_{max}^{EtOH} 225, 253, 305 nm (ε 27,250, 21,950, 22,050). ν_{max}^{KBr} (cm-'): 1770, 1638, 1575, 1500, 1490.

Oxyayanin-A and Derivatives

Oxyayanin-A (*VIIIa*). Orange needles, m.p. 225–227°, (lit.[15] 229-230). λ_{max}^{EtOH} 257, 291, 350 (ε 43,250 14,000, 20,700), $\lambda_{max}^{EtOH+NaOH}$ 268, 358, $\lambda_{max}^{EtOH+NaOH+HCl}$ 254,294, $\lambda_{max}^{EtOH+AlCl_3}$ 270, 314,400, no alteration with NaOAc, H_3BO_3 +NaOAc. ν_{max}^{KBr} (cm-'): 3386, 3193, 1653, 1595, 1494.

S-0-Methyloxyayanin-A (*VIIIb*). White crystals, m.p. 258-260". λ_{max}^{EtOH} 253, 345 (ε 43,000, 27,650), $\lambda_{max}^{EtOH+NaOH}$ 250, 315, $\lambda_{max}^{EtOH+NaOH+HCl}$ 248,295 nm, no alteration with NaOAc, H_3BO_3+NaOAc, $AlCl_3$. ν_{max}^{KBr} (cm^{-1}): 3340, 3244, 1626, 1515, 1499.

Oxyuyanin-A trimethyl ether (*VIIIc*). White crystals from benzene-light petroleum and methanol, m.p. 189–191°, (lit." 193–195°). λ_{max}^{EtOH} 245, 295, 324 nm (ε 20,600, 7800, 10,050). ν_{max}^{KBr} (cm-'): 1634, 1626, 1514. Methylation of S-0-methyloxyayanin-A with Me_2SO_4 also afforded oxyayanin-A trimethyl ether.

S-0-Methylaxyayanin-A diacetate (*VIIId*). White crystals from light petroleum-benzene, m.p.188–190°. ν_{max}^{KBr} (cm^{-1}): 1769, 1641 1612, 1577, 1515.

Oxyayanin-A triacetate (*VIIIe*). White crystals from light petroleum-benzene, m.p.181–183°, (lit.15 183-184"). ν_{max}^{KBr} 1762, 1626, 1569, 1493, 1439, cm-r.

Other Apuleia *Constituents*

Ayanin (X). White needles, m.p. 172–174°, (lit.[20] 172-174"). λ_{max}^{EtOH} 255, 265, 295, 353 (ε 8500, 7000, 3650, 7550), $\lambda_{max}^{EtOH+NaOH}$ 270, 290, 380, $\lambda_{max}^{EtOH+NaOH+HCl}$ 255, 265, 295, 353, $\lambda_{max}^{EtOH+AlCl_3}$ 265, 275, 295, 353, 393 nm, no alteration with NaOAc, H_3BO_3 + NaOAc. ν_{max}^{KBr} (cm-'): 3623, 3077, 1667, 1613, 1531, 1506.

Leiocarpin (XVI). White rectangular plates, m.p. 98-100". λ_{max}^{EtOH} 230, 290, 308 nm (ε 14,000, 4200, 4400); no alteration with NaOH. ν_{max}^{KBr} (cm-'): 1639, 1613, 1587, 1504. NMR spectrum (CCL, τ): 2.76 (d, *J* 8·0 Hz, H-l), 3.31 (s, H-7), 3.34 (d, *J* 9.4 Hz, H-4'), 3.48 (d, *J* 8·0 Hz, H-2), 3.58 (s, H-10), 402 (s, OCH_2O), 4.48 (d, *J* 9·4 Hz, H-3'), 4·58 (d, *J* 6·0 Hz, H-l la), 5·63–5·93 (m, H-6eq.), 6·18–6·73 (m, H-6ax., H-6a), 8·50 (s, $2CH_3$). Mass spectra: M^+ 350 (80%), m/e (%) 336 (23), 335 (100), 321 (5), 317 (5), 185 (12), 173 (19), 168 (7), 167·5 (24), 167 (6). ORD in EtOH (c 0·196, 440–240 nm): ϕ_{435} 0, ϕ_{326} −11,200, ϕ_{310} 0, ϕ_{294} + 16,400, ϕ_{250} + 31,400.

β-Sitosterol. White crystals, m.p. 134–136°, $[\alpha]_D^{20°}$ -37".

(+)-Pinitol. White crystals, m.p. 185-187" (lit.[40] 185–186°), $[\alpha]_D^{20°}$ + 60" (c 0.89, MeOH) (lit.[40] + 65").

Selective Demethylation of Apuleia *Constituents*

Formation of 5-O-demethylapulein (Ib). A mixture of apulein (100 mg) and 20% aq. HCl (70 ml), was heated on a steam with occasional agitation for 30 min, cooled to room temp., diluted with water and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with water, dried and evaporated. The residue was separated by thick layer chromatography (silica, benzene-EtOAc 1:1) into apuiein and 5-*O*-demethyl-apulein.

Formation of oxyayanin-A (VIIIa). 5-0-Methyloxyayanin-A (20 mg) was treated with HCl by the procedure described above (1 hr heating on the steam bath), yielding, besides starting material, oxyayanin-A.

Oxidative Demethylation of Apulein

Formation of the Quinone IV. A mixture of apulein (344 mg) and HNO_3 (d 1·40, 8·6 ml) was shaken, with occasional warming on the steam bath, for 30 min, left for an additional 30 min in an ice bath, poured into water, and extracted with $CHCl_3$. The $CHCl_3$ solution was dried and evaporated, yielding a deep orange solid (210 mg) m.p. 120-l 30". ν_{max}^{KBr} (cm^{-1}): 1696, 1660, 1654, 1605, 1542, 1508.

Formation of 2',5,5',6-*tetrahydroxy*-3,4'7-*trimethoxyflavone* (*Ih*). A mixture of the quinone IV (200 mg), Na_2SO_3 (1 g), and anhydrous AcOH (4 ml) was heated on the steam bath for 3 min, cooled to room temp., diluted with water and extracted with $CHCl_3$. The $CHCl_3$ solution was dried and evaporated, yielding a yellowish solid, m.p. 250-260". ν_{max}^{KBr} (cm^{-1}): 3369–3138, 1639, 1590, 1563.

Formation of 2',5,5',6-*tetraacetoxy*-3,4',7-*trimethoxyflavone* (*Ie*). The quinol Ih was acetylated without purification, the crude product being purified by passage through a silica column (eluant : benzene-AcOEt 1:1) when it crystallized from benzene as white needles (41 mg), m.p. 213-215". λ_{max}^{EtOH} 230, 250, 306 nm (ε 15,650, 10,900, 13,050). ν_{max}^{KBr} (cm-'): 1778, 1640, 1633, 1615, 1582, 1515, 1492. Mass spectrum: M^+ 544 (8%); *m/e* (%) 502 (8), 461 (25), 460 (100), 459 (8), 418 (17), 417 (8), 401 (7), 388 (5), 387 (21), 377 (5), 376 (26), 375 (25), 359 (10), 345 (17), 231 (5), 230 (10), 195 (5), 194 (8), 179 (11), 167 (6), 153 (8), 151 (9), 149 (8), 137 (7). 135 (IO), 129 (5), 122 (8), 115 (6), 111 (6), 109 (6), 107 (8), 106 (5), 105 (19), 104 (6), 103 (6).

Alkaline Hydrolysis of Apuleia *Constituents and of Their Derivatives*

Formation of 2,4,5-*trimethoxybenzoic acid and of* 6'-*hydroxy*-2,2'3',4'-*tetramethoxyacetophenone. A* mixture of apulein dimethyl ether (If, 150 mg), 50% aq. KOH 5 ml), and EtOH (10 ml) was heated (N_2 atm) on a steam bath for 8 hr. The ethanol was removed by distillation and simultaneously substituted with water. The aqueous solution was acidified (2N HCl) and extracted with ether. The ether solution was extracted successively with aq. $NaHCO_3$ (conc.) and with aq. NaOH (3%). The $NaHCO_3$ solution was acidified (2N HCl) and extracted with $CHCl_3$. The $CHCl_3$ solution was evaporated, and the residue purified by filtration through silica (eluant: $CHCl_3$) and crystallization from benezene–light petroleum yielding white needles of 2,4,5-trimethoxybenzoic acid (14 mg), m.p. and mixed m.p. with an authentic sample 139–141°, (lit.[15] 143-144"); ν_{max}^{KBr} (cm-'): 3400, 3225, 2500 broad, 1719, 1613, 1590, 1518. The NaOH solution was acidified (2N HCl) and extracted with $CHCl_3$. The $CHCl_3$ solution was evaporated, and the residue purified by filtration through silica (eluant: $CHCl_3$) and crystallization from light petroleum yielding white needles of 6'-hydroxy-2,2',3',4'-tetramethoxyacetophenone (5 mg), m.p. and mixed m.p. with an authentic sample 69–71°, (lit.[14] 70-71"); λ_{max}^{EtOH} (nm): 216, 230, 284 (ε 5550, 4550, 5400); $\lambda_{max}^{EtOH+NaOH}$ 242, 282, 365, $\lambda_{max}^{EtOH+AlCl_3}$ 216, 234, 290, 305 sh. ν_{max}^{KBr} (cm-i): 3421, 1634, 1613, 1585, 1492. Mass spectrum: M^+ 256 (14%), *m/e* (%) 212 (12), 211 (100), 210 (6), 196 (6), 195 (6), 184 (7), 169 (10), 168 (6), 167 (8), 153 (5), 149 (5).

[40] W. KARRER, *Konstitution und Vorkommen der Organischen Pflanzenstoffe*, p. 117, Birkhluser Verlag, Base1 (1958).

***Formation of** 4-methoxy-2,5-diethoxybenzoic acid and of 6′-hydroxy-2,2′,3′,4′-tetramethoxyacetophenone.* A mixture of apulein diethyl ether (Ig, 40 mg) and 10% KOH in EtOH (10 ml) was heated (N_2 atm) on a steam bath for 10 hr. Treatment as described above, afforded an acidic and a phenolic fraction. The acidic fraction yielded, upon sublimation, 4-methoxy-2,5-diethoxybenzoic acid (2 mg), m.p. and mixed m.p. with an authentic sample 152–154°, (lit.[15] 155-156"); λ_{max}^{KBr} (cm-'): 2500 broad, 1677, 1651, 1610, 1600, 1511. Mass spectrum: M^+ 240 (63%), *m/e* (%) 212 (6), 211 (11), 196 (12), 195 (10), 194 (48), 184 (8), 183 (18), 168 (10), 167 (22), 166 (100), 165 (72), 151 (7), 144 (22), 139, (32), 138 (13), 137 (23), 135 (12), 128 (6), 12.5 (15), 123 (23), 122 (5), 119 (7), 115 (6), 111 (32), 110 (7), 109 (18), 108 (6), 107 (12), 105 (16), 103 (6). In the phenolic fraction, the presence of 6′-hydroxy-2,2′,3′,4′-tetramethoxyacetophenone was demonstrated by TLC (silica, benzene-AcOEt 1 :1).

***Formation of** 6-hydroxy-2,3,4-trimethoxybenzoic acid and of 6′-hydroxy-2,2′,3′,4′-tetramethoxyacetophenone.* A mixture of apulein (Ia, 20 mg), 20% NaOH (in MeOH–water 1 :1 (1.5 ml)) was heated under reflux on a steam bath during 4 hr. Treatment as described above, afforded an acidic and a phenolic fraction. The acidic fraction yielded, upon sublimation, 6-hydroxy-2,3,4-trimethoxybenzoic acid (2 mg), m.p. 113-115". ν_{max}^{KBr} (cm-'): 3323, 3168, 1679, 1613, 1586, 1493. Mass spectrum: M^+ 228 (41%), m/e (%) 211 (15), 210 (100), 196 (7), 195 (65), 184 (30), 169 (34), 168 (7), 167 (49), 141 (9), 139 (6), 111 (7), 105 (5), 83 (6), 69 (40), 66 (7), 55 (5), 53 (18). The NaOH solution was acidified and extracted with $CHCl_3$. In the phenolic fraction, the presence of 6′-hydroxy-2,2′,3′,4′-tetramethoxyacetophenone was demonstrated by TLC.

***Formation of** 2,3,4-trimethoxybenzoic acid and of 6′hydroxy-2,2′,3′,4′-tetramethoxyacetophenone.* A mixture of apuleisin tetramethyl ether (Vb, 80 mg), KOH (1.5 g), EtOH (11 ml) and water (3 ml) was heated under reflux until TLC indicated complete absence of starting material in the reaction mixture. Treatment as described above, afforded an acidic and a phenolic fraction. The acidic fraction was crystallized from light petroleum benzene, yielding 2,3,4-trimethoxybenzoic acid (10 mg), m.p. 99-100" (lit."' 100"). λ_{max}^{KBr} (cm-'): 3021, 2600 broad, 1681, 1590, 1493. NMR spectrum [$(CD_3)_2CO$, τ): 6.28 (s, OCH_3), 6·10 (s, OCH_3), 6·04 (s, OCH_3), 3·11 (d, J 8·4 Hz, H-5), 2.38 (d, J 8·4 Hz, H-6). The phenolic fraction was crystallized from light petroleum, yielding 6′-hydroxy-2,2′,3′,4′-tetramethoxyacetophenone, m.p. and mixed m.p. with an authentic sample 67-69" (lit."' 70-71").

Hydrogenolysis of Leiocarpin

***Formation of** (3S)-2′-hydroxy-4′,5′-methylenedioxy-6″,6″-dimethylpyrano-(2″,3″:7,8)-isoflavan* (XV). A suspension of 10 % Pd/C (250 mg) in AcOH (15 ml) was saturated with hydrogen at room temp. and pressure. A solution of leiocarpin (100 mg) in EtOH (5 ml) was added and hydrogenation continued to the disappearance (TLC) of starting material. The mixture was filtered, evaporated and the residue crystallized from CCl_4, yielding white crystals (72 mg), m.p. 184-186". λ_{max}^{EtOH} 230, 288, 300 (ϵ 12,750, 6000, 6550); $\lambda_{max}^{EtOH+NaOH}$ 227, 280, 317 nm. Gibbs test: negative. ν_{max}^{KBr} (cm-'): 3420, 1610, 1590, 1510, 1490. NMR spectrum in MeCN (τ): 3.16 (d, J 8·6 Hz, H-5), 3.31 (s, H-6'), 3.49 (s, H-3'), 3·70 (d, J 8·6 Hz, H-6), 4.12 (s, OCH_2O). Mass spectrum: M^+ 354 (59%), *m/e* (%) 216 (7), 192 (16), 191 (98), 177 (5), 176 (22), 175 (6), 165 (15), 164 (100), 152 (6), 151 (28), 150 (5), 149.5 (8), 149 (12), 147 (12), 137 (5), 136 (16), 135 (47), 133 (15). ORD in EtOH (c 0.18): ϕ_{326} 0, ϕ_{298} −4520, ϕ_{289} 0, ϕ_{260} + 4280, ϕ_{250} + 8650.

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[41] K. R. HARGREAVES, A. MCGOOKIN and A. ROBERTSON, *J. Appl. Chem. 8,273* (1958).