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THE CHEMISTRY OF THE EUPHORBIACEAE—XIII[1]

FLAVONES AND MINOR DITERPENES FROM RICINOCARPUS MURICATUS

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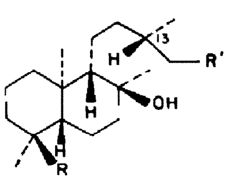
Abstract—The new diterpene acids, eperu-8(20)-en-15,18-dioic acid (IIIa), 15-hydroxyeperu-8(20)-en-18-oic acid (IIId) and the $\Delta^{\alpha\beta}$-butenolide (Va) have been isolated from *Ricinocarpus muricatus*, which has also yielded the new flavone pigments 5,3′,4′-trihydroxy-3,7,8-trimethoxyflavone (IXc) and 5,7,3′,4′-tetrahydroxy-3,8-dimethoxyflavone (IXd).

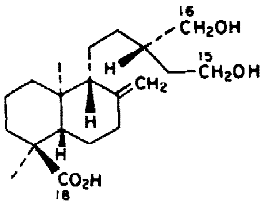
In part XI[2] we described the structural chemistry of eperuane-8β,15-diol (Ia), eperuane-8β,15,18-triol (Ib) and 15,16-dihydroxy-eperu-8(20)-en-18-oic acid (II) which were isolated from *Ricinocarpus muricatus* Muell. Arg. and we now wish to discuss some other compounds isolated from this source.

Repeated chromatography of the residual acidic fractions has provided the diacid (IIIa), the hydroxy-acid (IIId) and the γ-lactonic acid (Va) in yields of 0·06 %, 0·01 %, and 0·025 % respectively. The natural diacid (IIIa), $C_{20}H_{32}O_4$, readily formed a dicyclohexylamine salt and gave a dimethyl ester (IIIb) with diazomethane. The IR spectrum (CS_2) of IIIb showed absorption at 3078, 1641 and 890 cm^{-1} attributed to a vinylidene group and at 1735 and 1728 cm^{-1} due to the methyl ester functions. In the NMR spectrum of IIIa the vinylidene protons absorbed as broad singlets at 5·16 (1H) and 5·47τ (1H), and the chemical shifts (Table 1) of the two methyl groups at quaternary positions indicated[2,3] that if an eperuene skeleton was present the diacid should possess an equatorial 4-carboxyl group. The chemical shift of the C-13 methyl group was consistent with the presence of a 14-carboxyl group.[4] Reduction of the diacid (IIIa) with LAH gave eperu-8(20)-ene-15,18-diol (IIIc) identical with the sample derived[2] from eperuane-8β,15,18-triol (Ib). The diacid (IIIa) on titration, had pK$^{*}_{MCS}$ values[5] of 7·19 and 8·31.



I

	R	R′
(a)	CH_3	CH_2OH
(b)	CH_2OH	CH_2OH



II

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* Present Address: Department of Organic Chemistry, University of Sydney, Sydney, N.S.W.

[1] Part XII. P. R. Jefferies and T. G. Payne, *Austr. J. Chem.* **18**, (1965) in the press.

[2] C. A. Henrick and P. R. Jefferies, *Tetrahedron* **21**, 1175 (1965).

[3] C. A. Henrick and P. R. Jefferies, *Austr. J. Chem.* **17**, 915 (1964).

[4] C. A. Henrick, P. R. Jefferies and R. S. Rosich, *Tetrahedron Letters* No. 47, 3475 (1964).

[5] P. F. Sommer, C. Pascual, V. P. Arya and W. Simon, *Helv. Chim. Acta* **46**, 1734 (1963); C. Pascual and W. Simon, *Ibid.* **47**, 683 (1964).

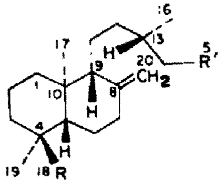
These values are in good agreement with those found (7·24, 8·35) for pinifolic acid[6] (IV), and differ significantly from those of dihydroagathene dicarboxylic acid (7·34, 8·85)[6] in which the 4-carboxyl group is axial. It is not surprising that the latter forms a monocyclohexylamine salt[6] under the usual conditions whereas both IIIa and IV give

TABLE 1. CHEMICAL SHIFTS OF PROTONS IN EPERUENE DERIVATIVES
(τ-values[7] in $CHCl_3$ or $CDCl_3$)

Compound	C-4 Methyl	C-10 Methyl	C-20 Hydrogens	C-16 Hydrogens	C-14 Hydrogen
IIIa	8·84	9·28	5·16, 5·47	9·01 (d_1)	—
IIIb	8·85	9·28	5·17, 5·48	9·05 (d_1)	—
IIId	8·84	9·28	5·15, 5·45	9·08 (d_1)	—
Va	8·83	9·25	5·09, 5·48	5·25 (d_2)	4·12 (t)
Vb	8·85	9·25	5·10, 5·46	5·23 (d_2)	4·15 (t)
VIb	8·79	9·22	—	—	—
VId	8·80	9·24	—	5·78 (2H)*	—
VII	8·77	8·94	—	6·16 (1H)*	—

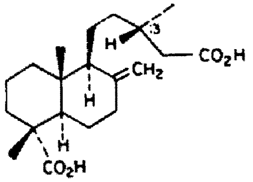
(d_1) = doublet (J, 6 c/s), (d_2) = doublet (J, 1·6 c/s), t = triplet (J, 1·6 c/s)
* singlet.

disalts. The diacid (IIIa) and the enantiomer of pinifolic acid (IV) are 13-epimers and the $[M]_D$ differences between IIIa and *enantio*-pinifolic acid (in EtOH) and between the corresponding dimethyl esters (in $CHCl_3$) are +34° and +19° respectively. These $\Delta[M]_D$ values are consistent with those found between corresponding derivatives of eperuic and *enantio*-labdanolic acids and support the assigned C-13 configuration in IIIa.[2]



III

	R	R'
(a)	CO_2H	CO_2H
(b)	CO_2Me	CO_2Me
(c)	CH_2OH	CH_2OH
(d)	CO_2H	CH_2OH



IV

The natural hydroxy-acid (IIId), $C_{20}H_{34}O_3$, which was characterized as the cyclohexylamine salt, had IR absorption (CS_2) bands at 3623 cm^{-1} (hydroxyl), 3082 and 890 cm^{-1} (vinylidene group), and 1742, 1692 cm^{-1} (carboxyl group). The NMR spectrum (Table 1) of IIId was consistent with a normal bicarbocyclic diterpene, the chemical shifts assigned to the 4- and 10-methyl groups indicating an equatorial 4-carboxyl group,[2] which was supported by a pK^*_{MCS} titration value[5] of 7·88 (calc 7·91).[5] The NMR

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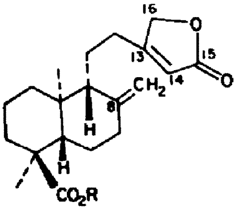
[6] C. Enzell and O. Theander, *Acta Chem. Scand.* **16**, 607 (1962).

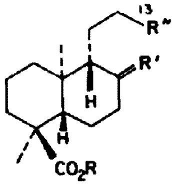
[7] G. V. D. Tiers, *J. Phys. Chem.* **62**, 1151 (1958).

spectrum of IIId showed a two-proton symmetrical triplet centred at 6·31τ (J, 6 c/s) attributable to the resonance of the C-15 hydrogens.[2] The chemical shift (Table 1) of the C-13 methyl group is also consistent with a 15-hydroxyl group.[2,4] Reduction of IIId with LAH gave the diol (IIIc) identical with the sample prepared above from IIIa, and oxidation of IIId with the Jones reagent[8] gave entirely the diacid (IIIa). In our experience with diterpenes, Jones oxidation of an equatorial 4-hydroxymethyl group gives mainly the corresponding 4-aldehyde.

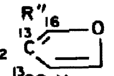


V

(a) R = H
(b) R = Me



VI

	R	R'	R''
(a)	H	CH_2	
(b)	H	O	$^{13}CO_2H$
(c)	Me	O	$\overset{13}{C}(=O)-\overset{16}{C}H_2O-C(=O)-CHO$
(d)	Me	O	$\overset{13}{C}(=O)-\overset{16}{C}H_2OH$

The α,β-unsaturated γ-lactone (Va), $C_{20}H_{28}O_4$, formed a methyl ester (Vb) with diazomethane and both Va and Vb gave a positive Legal test with alkaline sodium nitroprusside under the conditions outlined by Elderfield *et al.*[9] for β-substituted $\Delta^{\alpha\beta}$-butenolides. The ester (Vb) also gave an intense violet colour with *m*-dinitrobenzene in cold ethanolic alkali (Raymond test)[10] and the UV spectrum of Vb in ethanol showed a shoulder at ca. 214 mμ (log ε, 4·18)[9,11] with end absorption at 200 mμ, log ε 4·31. In cyclohexane solution however, only absorption at 198 mμ (log ε 4·41) could be observed.* The IR spectrum of Vb showed absorption at 1782 and 1749 cm^{-1} in carbon disulphide solution and at 1782 and 1748 cm^{-1} in chloroform, due to the $\Delta^{\alpha\beta}$-butenolide ring.[12] In the less polar solvents (CCl_4 and CS_2) the higher frequency band was the stronger of the two, while in chloroform and in a nujol mull the lower frequency band was much stronger. The IR behaviour of Vb is identical to that quoted[12] for β-n-hexyl-$\Delta^{\alpha\beta}$-butenolide. The ester (Vb) also showed a strong band in carbon disulphide at 880 cm^{-1} which has been assigned to the $\Delta^{\alpha\beta}$-butenolide ring,[12] and absorption at 3078, 1640 and 892 cm^{-1} due to the vinylidene group.

The NMR spectrum of Va showed only two methyl signals as singlets, the chemical shifts (Table 1) indicating an equatorial 4-carboxyl group in a normal bicarbocyclic

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* cf. Ref. 12.

[8] R. G. Curtis, I. Heilbron, E. R. H. Jones and G. F. Woods, *J. Chem. Soc.* 457 (1953); see also C. Djerassi, R. R. Engle and A. Bowers, *J. Org. Chem.* **21**, 1547 (1956).

[9] W. D. Paist, E. R. Blout, F. C. Uhle and R. C. Elderfield, *J. Org. Chem.* **6**, 273 (1941).

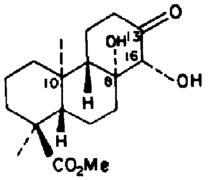
[10] L. F. Fieser and M. Fieser, *Steroids*, Chap. 20; p. 734. Reinhold, New York (1959).

[11] L. Dorfman, *Chem. Rev.* **53**, 95 (1953).

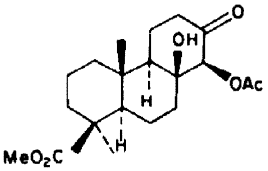
[12] R. N. Jones, C. L. Angell, T. Ito and R. J. D. Smith, *Canad. J. Chem.* **37**, 2007 (1959).

skeleton.[2] The C-16 hydrogens absorbed as a clear doublet* (2H) at 5·25τ (J, 1·6 c/s) and the C-14 proton absorbed as a triplet (1H) at 4·12τ (J, 1·6 c/s), the chemical shift of the latter being consistent with a vinylic proton α to the carbonyl group.† The allylic coupling constant (1·6 c/s) and the chemical shifts of the 14- and 16-hydrogens in Va agree well with the values observed for steroid cardenolides.[14,15]

Ozonolysis of Va followed by oxidation with alkaline hydrogen peroxide gave the keto-dicarboxylic acid (VIb)[16] identical with a sample obtained by the same procedure from the known polyalthic acid (VIa).[16] Ozonolysis of the methyl ester (Vb) at −78° followed by zinc-acetic acid reduction and mild hydrolysis[17] of the intermediate ester (VIc) gave a neutral product, the NMR spectrum (Table 1) of which showed it to be virtually pure diketo-alcohol (VId). However, after manipulation the aldol condensation product (VII) was obtained, the stereochemistry of the latter being based on the analogous product VIII obtained by ozonolysis of diacetyl methyl sciadopate.[18] The IR spectrum (CS$_2$) of VII showed only intramolecularly hydrogen bonded hydroxyl absorption at 3553 and 3457 cm^{-1}.



VII



VIII

In the NMR spectrum (Table 1) of VId the 10-methyl group absorbs at the same position as that in VIb,‡ however in VII the 10-methyl group is considerably deshielded and part of this downfield shift of the 10-CH$_3$ signal could be due to the introduction of the 8α-hydroxyl group which is in a 1,3-diaxial relationship to the 10-methyl group.[19]

The γ-lactone (Va) is the first diterpene reported to contain a β-substituted $\Delta^{\alpha\beta}$ butenolide of the type found in the cardenolides. Other examples of diterpenes containing an α,β-unsaturated γ-lactone group are andrographolide,[20] α- and β-levantenolide,[21] and eremolactone.[22]

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* cf. Ref. 15.

† The corresponding β-proton in $\Delta^{\alpha\beta}$-butenolide absorbs at 2·37τ.[13]

‡ cf. Ref. 2.

[13] N. S. Bhacca, L. F. Johnson, J. N. Shoolery, *NMR Spectra Catalog*, Spectrum No. 51, Varian Associates, Palo Alto, California (1962).

[14] R. M. Carman, R. G. Coombe and T. R. Watson, *Austr. J. Chem.* 17, 573 (1964); D. J. Collins, J. J. Hobbs and S. Sternhell, *Ibid.* 16, 1030 (1963).

[15] N. S. Bhacca and D. H. Williams, *Applications of NMR Spectroscopy in Organic Chemistry* p. 45. Holden-Day, San Francisco (1964).

[16] K. W. Gopinath, T. R. Govindachari, P. C. Parthasarathy and N. Viswanathan, *Helv. Chim. Acta* 44, 1040 (1961).

[17] A. Buzas and T. Reichstein, *Helv. Chim. Acta* 31, 84 (1948); H. Kündig-Hegedüs and O. Schindler, *Ibid.* 39, 904 (1956).

[18] M. Sumimoto, Y. Tanaka and K. Matsufuji, *Tetrahedron* 20, 1427 (1964).

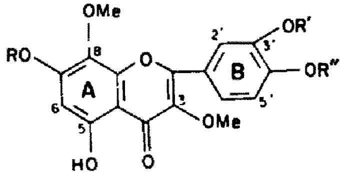
[19] K. Tori and E. Kondo, *Tetrahedron Letters* No. 10, 645 (1963).

[20] M. P. Cava, B. Weinstein, W. R. Chan, L. J. Haynes and L. F. Johnson, *Chem. & Ind.* 167 (1963); W. R. Chan, C. Willis, M. P. Cava and R. P. Stein, *Ibid.* 495 (1963).

[21] J. A. Giles and J. N. Schumacher, *Tetrahedron* 14, 246 (1961).

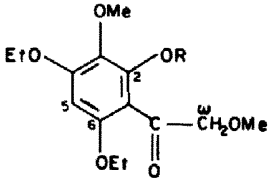
[22] A. J. Birch, J. Grimshaw and J. P. Turnbull, *J. Chem. Soc.* 2412 (1963).

In the process of isolation of the diterpenoids, three flavonoid pigments were obtained. One of these, 5,3′-dihydroxy-3,7,8,4′-tetramethoxyflavone (IXb), had been isolated previously from *Ricinocarpus stylosus.*[23] The second pigment (IXc), $C_{15}H_7O_5(OCH_3)_3$, was isolated as the triacetate. One of the hydroxyl groups was recognized as the 5-hydroxyl by partial methylation of the pigment with diazomethane to gossypetin pentamethyl ether (IXa), which also established the oxygenation pattern. Ethylation of the natural pigment with excess diethyl sulphate gave a triethyl ether which on alkaline degradation gave 3,4-diethoxybenzoic acid (protocatechuic acid diethyl ether), thus locating the other hydroxyl groups.



IX

	R	R′	R″
(a)	Me	Me	Me
(b)	Me	H	Me
(c)	Me	H	H
(d)	H	H	H



X

(a) R = H
(b) R = CH_3CH_2

Separation of the sodium bicarbonate soluble fractions provided a third flavonoid pigment (IXd), $C_{15}H_8O_6(OCH_3)_2$, which was isolated as the tetra-acetate. Alkaline degradation of the tetraethyl ether gave 3,4-diethoxybenzoic acid and 2-hydroxy-4,6-diethoxy-ω,3-dimethoxyacetophenone (Xa),[24] converted to (Xb)[24] by ethylation, thus establishing this flavone as a new gossypetin derivative isomeric with limocitrin (3,5,7,4′-tetrahydroxy-8,3′-dimethyoxyflavone).[25]

The structures of the new flavonoid pigments (IXc and IXd) were originally suggested by an examination of the NMR spectra (Table 2) of their derived acetates. The spectra showed the B-ring aromatic proton signals as first order ABC patterns and the 6-H signals as singlets. The chemical shift of the 6-H in the acetates of IXb and IXc indicated a 5-acetoxyl group in the latter,[23] and this was supported by an acetoxy methyl signal at 7·54τ.[23,26] The downfield shift of the 6-H signal in the spectrum of the acetate of IXd relative to that in the acetate of IXb indicated hydroxyl groups at the 5- and 7-positions in IXd.[23,26] Similarly, the downfield shift of the 5′-H signals in the spectra of the acetates of IXc and IXd relative to that in the acetate of IXb indicated the presence of 3′,4′-dihydroxy groups in IXc and IXd.[23]

The UV spectra (Table 3) of the new flavones IXc and IXd are consistent with the established structures. The large bathochromic shift of the long wavelength band, without a decrease in its relative intensity, observed on making the solutions of IXc and IXd alkaline indicates a 4′-hydroxyl group.[27] The free 7-hydroxyl group in IXd is

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[23] C. A. Henrick and P. R. Jefferies, *Austr. J. Chem.* **17**, 934 (1964).

[24] R. M. Dawson, C. A. Henrick, P. R. Jefferies and E. J. Middleton, *Austr. J. Chem.* in the press.

[25] R. M. Horowitz and B. Gentili, *J. Org. Chem.* **26**, 2899 (1961).

[26] J. Massicot and J-P Marthe, *Bull. Soc. Chim. Fr.* 1962 (1962); J. Massicot, J-P. Marthe and S. Heitz, *Ibid.* 2712 (1963).

[27] L. Jurd, *The Chemistry of Flavonoid Compounds* (Edited by T. A. Geissman), Chap. 5; Pergamon Press, Oxford (1962).

Table 2. Chemical shifts of protons in flavone acetates
(τ-values[7] in $CDCl_3$)

Compound	A-Ring	B-Ring			Methoxyls	Acetoxyls
	6-H	2'-H	6'-H	5'-H		
Diacetate of IXb[*]	3·31 S	2·05 D_m	1·88 Q	2·87 D_o	6·02, 6·04, 6·09, 6·16	5-OAc 7·54 3'-OAc 7·65
Triacetate of IXc	3·31 S	1·90 D_m	1·89 Q	2·61 D_o	6·04(2), 6·14	5-OAc 7·54 3'-OAc 7·66 4'-OAc 7·68
Tetra-acetate of IXd	3·19 S	1·97 D_m	1·95 Q	2·64 D_o	6·03, 6·13	5-OAc 7·56 3'-OAc 7·66 4'-OAc 7·69 7-OAc 7·71

S, singlet; D_m, doublet (J_m 2·0 c/s); Q, quartet (J_m 2·0 c/s, J_o 9·0 c/s); D_o, doublet (J_o 9·0 c/s).

Coupling constants were derived by first order analysis only.

Table 3. UV spectra of flavones
(λ_{max} in mμ)

Compound	EtOH		NaOH-EtOH[b]		EtOH-NaOAc[c]		EtOH-NaOAc-H_3BO_3[d]	
	λ_{max}	log ε	λ_{max}	log ε	λ_{max}	log ε	λ_{max}	log ε
IXc	262	4·34	269	4·33	267	4·33	264	4·39
	271[a]	4·32						
	374	4·19	408	4·29	424	4·25	387	4·26
IXd	267[a]	4·35	276[a]	4·47				
	276	4·40	282	4·48	284	4·48	265	4·45
	338[a]	4·15	343[a]	4·05	329	4·11		
	373	4·21	420	4·44	410	4·24	385	4·32

[a] shoulder; [b] 1 drop of 4% NaOH aq in 2·5 ml cell; [c] EtOH saturated with dry sodium acetate; [d] EtOH saturated with dry sodium acetate and boric acid.

indicated by the bathochromic shift of the short wavelength band in the presence of fused sodium acetate in absolute ethanol.[25,28] The presence of a free *o*-dihydroxyl group in IXc and IXd is supported by the bathochromic shift of the long wavelength band in the presence of boric acid and sodium acetate.[28,29]

The isolation of the three gossypetin methyl ethers (IXb, IXc and IXd) from *R. muricatus* is of interest in view of the occurrence of IXb, IXa and 5,4'-dihydroxy-3,7,8-trimethoxyflavone in *R. stylosus*.[23] The synthesis of 5,8,3',4'-tetrahydroxy-3,7-dimethoxyflavone by partial methylation of gossypin has recently been published.[30] A similar partial methylation of gossypitrin (7-glucoside of gossypetin) with dimethyl sulphate in the presence of borax and alkali, followed by hydrolysis would afford IXd.

[*] Ref. 23.

[28] L. Jurd and R. M. Horowitz, *J. Org. Chem.* **22**, 1618 (1957); see also B. Gentili and R. M. Horowitz, *Tetrahedron* **20**, 2313 (1964).

[29] L. Jurd, *Arch. Biochem. and Biophys.* **63**, 376 (1956).

[30] N. K. Anand, S. R. Gupta, A. C. Jain, S. K. Mathur, K. S. Pankajamani and T. R. Seshadri, *J. Sci. Industr. Res. India* **21B**, 322 (1962).

EXPERIMENTAL

Analyses were carried out by the Australian Microanalytical Service, Melbourne. Rotations were determined in $CHCl_3$ unless otherwise stated, and at room temp (22°). All identities were confirmed by comparison of IR spectra measured with Perkin–Elmer Infracords 137 and 137G for the ranges 1300–670 cm^{-1} and 4000–1300 cm^{-1} respectively. All solution IR spectra quoted were measured with a Perkin–Elmer model 521 spectrophotometer (grating). The NMR spectra were measured with a Varian A-60 spectrometer (60 Mc) for $CDCl_3$ or $CHCl_3$ solutions containing tetramethylsilane as internal reference. All chemical shifts are quoted on the τ-scale.[7] Light petroleum had b.p. 55–60°. M.ps are uncorrected and were determined on a Kofler block unless designated (cap). The latter values are for sealed evacuated capillaries. UV absorption spectra were determined for EtOH solutions, unless otherwise stated, with a Perkin–Elmer 137 UV.

Isolation of the acidic constituents of Ricinocarpus muricatus

Dried crushed plant (12 kg) collected 15 miles south of Norseman, Western Australia, in May 1964 was extracted with cold ether. The extract was washed thoroughly with 8% $NaHCO_3$ aq and then 5% NaOH aq. Isolation of the acids from the former solution with 5% HCl aq and ether gave a residue which readily crystallized. Washing with a little ether gave 15,16-dihydroxy-eperu-8(20)-en-18-oic acid (II)[8] as plates (25 g) m.p. and mixed m.p. 152–153° after crystallization from acetone. The ether soluble portion slowly crystallized. Washing with a little ether gave an insoluble precipitate (1·5 g) which was acetylated with acetic anhydride and perchloric acid catalyst. The crude acetate was dissolved in benzene and the solution filtered through a column of neutral alumina (Act III). The eluate was crystallized from MeOH to afford needles (0·6 g) of 5,7,3′,4′-*tetra-acetoxy*-3,8-*dimethoxyflavone*, m.p. 149–150°, λ_{max} mμ (log ε) = 259 (4·57), 304 (4·24), 328 (4·26). (Found: C, 58·3; H, 4·3; CH_3O, 11·7. $C_{25}H_{22}O_{12}$ requires: C, 58·4; H, 4·3; 2 × CH_3O, 12·1%.) The acetate was photosensitive, rapidly turning orange on exposure to light. The residual ether soluble dark resin (30 g) was dissolved in ether and passed through a column of activated charcoal (75 g), to give two main groups of fractions, A and B. The early fractions (A; 5 g) were combined and rechromatographed in ether solution on activated charcoal (100 g). Fractions 8 to 10 (0·50 g) were combined and recrystallized from benzene–light petroleum to give *eperu*-8(20)-*en*-15,18-*dioic acid* (IIIa; 0·30 g) as dimorphic needles and prisms. m.p. 151–152° and 158–159°, $[\alpha]_D$ −36° (*c*, 5·4 EtOH), ν_{max}^{Nujol} = 3082, 1710, 1645, 890 cm^{-1}, pK^*_{MCS} 7·19, 8·31. (Found: C, 71·3; H, 9·5; O, 19·6. $C_{20}H_{32}O_4$ requires: C, 71·4; H, 9·6; O, 19·0%.) Fractions B (13 g) above were rechromatographed on silicic acid (Act II; 200 g). Elution with ether–benzene (1:9 and 1:3) gave a resin (5 g) which crystallized from benzene–light petroleum as needles (3 g) of the *γ-lactone acid* (Va), m.p. 190–191°, $[\alpha]_D$ −61° (*c*, 4·5 EtOH), λ_{max} = shoulder at ca. 214 mμ (log ε 4·18); end absorption at 200 mμ (log ε 4·32), $\nu_{max}^{CCl_4}$ = 3080, 1784, 1752, 1694, 1642, 890 cm^{-1}. (Found: C, 72·2; H, 8·6; O, 19·7. $C_{20}H_{28}O_4$ requires: C, 72·3; H, 8·5; O, 19·3%.) The α,β-unsaturated γ-lactone showed a positive Legal test.[9] Further elution of the silicic acid column with ether and crystallization of the eluate from acetone gave the dihydroxy-acid (II) as plates (4·5 g).

The 5% NaOH aq extract was acidified with 5% HCl aq, the acids extracted into ether, which was then rewashed with 8% $NaHCO_3$ aq, 5% Na_2CO_3 aq and 5% NaOH aq solutions. The acids from the bicarbonate extract were recovered with acid and ether to give a resin which slowly crystallized. Washing with a little ether gave an insoluble pigment (2·6 g) which was acetylated with acetic anhydride–perchloric acid, the acetate dissolved in benzene and filtered through neutral alumina (Act III; 20 g). Crystallization from MeOH gave the tetra-acetate of IXd as needles (1·7 g) m.p. and mixed m.p. 149–150°. The residual ether soluble resin (45 g) was chromatographed in ether on activated charcoal (100 g) to give two groups of fractions. The last fractions were crystallized from acetone to give II (6 g). The first three fractions (15 g) were combined and rechromatographed in ether on activated charcoal (300 g). Fractions 10–15 (10 g) were crystallized from benzene–light petroleum to give the diacid (IIIa; 7 g).

Recovery of the acids from the 5% Na_2CO_3 aq extract above gave a residue which soon crystallized. Washing with ether gave an insoluble pigment mixture (16 g) and an ether soluble residue (80 g). The former was dissolved in MeOH and then fractionally precipitated with water. The first two crops (9 g) were combined, acetylated with acetic anhydride–perchloric acid, the acetate dissolved in benzene and the solution filtered through neutral alumina (Act III; 20 g). Crystallization of the eluate from EtOH gave needles (7·0 g) of 5,3′,4′-*triacetoxy*-3,7,8-*trimethoxyflavone*, m.p. 184–185°, λ_{max} mμ (log ε) = 264 (4·49), 308 (4·28), 333 (4·26). (Found: C, 59·5; H, 4·9; CH_3O, 18·7. $C_{24}H_{22}O_{11}$ requires: C, 59·3; H, 4·6; 3 × CH_3O, 19·1%.) The last two crops (2·5 g) from the above fractional crystallization, were acetylated, the acetate dissolved in benzene and passed through neutral alumina (Act III). Crystallization from MeOH gave the tetra-acetate of IXd as needles (0·9 g), m.p. and mixed m.p. 149–150°. Half (40 g) of the ether soluble residue above was dissolved in ether and the solution filtered through activated charcoal (100 g). The first three fractions (12 g) were combined and chromatographed on silicic acid (Act II; 200 g). Elution with ether–benzene (1:3) gave a resin (7·1 g) which was methylated with diazomethane in ether and the esters chromatographed on neutral alumina (Act III; 250 g). Elution with benzene–light petroleum (1:1) and benzene gave an oil (1·55 g) which was saponified by dissolving it in ethylene glycol (45 ml) and EtOH (5 ml), adding KOH (10 g) and water (5 ml), and heating the mixture under reflux for 6 hr. Dilution with water, acidification and isolation with ether gave a resin (1·45 g) which crystallized on adding a little ether. Recrystallization from benzene–light petroleum followed by aqueous MeOH gave needles (0·55 g) of 15-*hydroxy-eperu*-8(20)-*en*-18-*oic acid* (IIId), m.p. 136–137°, $[\alpha]_D$ −51° (*c*, 1·1 EtOH), $\nu_{max}^{CS_2}$ = 3623, 3082, 1742, 1692, 890 cm^{-1}, pK^*_{MCS} 7·88. (Found: C, 74·2; H, 10·5; O, 15·8. $C_{20}H_{34}O_3$ requires: C, 74·5; H, 10·6; O, 14·9%.) The isolation of this acid was directed by the NMR spectra of the chromatogram fractions.

The fraction soluble in 5% NaOH aq was recovered with 5% HCl aq and ether, and the resin allowed to crystallize. Washing with ether gave an insoluble pigment fraction (11 g) which was boiled with MeOH (200 ml) and the mixture allowed to cool. The insoluble material (4·5 g) was acetylated with acetic anhydride–perchloric acid, the acetate dissolved in benzene and filtered through a plug of neutral alumina (Act III). Crystallization from EtOH gave needles (4 g) of 5,3′-diacetoxy-3,7,8,4′-tetra-methoxyflavone,[23] m.p. and mixed m.p. 179–180°. Water was added to the MeOH mother liquor above and several crops of crystals were obtained. The last crop (1·2 g) was acetylated and the acetate recrystallized from EtOH to give the triacetate of IXc as needles (0·5 g), m.p. and mixed m.p. 183–185°.

Derivatives of eperu-8(20)-*en*-15,18-*dioic acid* (IIIa)

The dimethyl ester (IIIb), prepared with diazomethane in ether at 0° in the usual way, was filtered through a column of neutral alumina (Act IV) in ether to give an oil, $[\alpha]_D$ −32° (*c*, 6·0), $\nu_{max}^{CS_2}$ = 3078, 1735, 1728, 1641, 890 cm^{-1}. (Found: C, 72·4; H, 10·1. $C_{22}H_{36}O_4$ requires: C, 72·5; H, 10·0%.)

The dicyclohexylamine salt of IIIa, prepared in hot ethyl acetate, was recrystallized from MeOH–ethyl acetate to give small needles, m.p. 212–215° (cap, dec), $[\alpha]_D$ −33° (*c*, 0·9 EtOH), ν_{max}^{Nujol} = 3080, 2230, 1630, 884 cm^{-1}. (Found: C, 71·5; H, 11·0; N, 5·3. $C_{32}H_{58}O_4N_2$ requires: C, 71·9; H, 10·9; N, 5·2%.)

Reduction of eperu-8(20)-*en*-15,18-*dioic acid* (IIIa)

The diacid (0·92 g) in ether (100 ml) was heated under reflux with LAH (2 g) for 6 hr. The excess reagent was destroyed with ice-water and the neutral product (0·85 g) isolated with ether. Recrystallization from benzene–light petroleum gave needles (0·70 g) of eperu-8(20)-ene-15,18-diol (IIIc), m.p. and mixed m.p. 101–102°. (Lit.[3] m.p. 101–102°).

Derivatives of 15-*hydroxyeperu*-8(20)-*en*-18-*oic acid* (IIId)

The cyclohexylamine salt of IIId, prepared in hot ethyl acetate–light petroleum, crystallized from the same solvent mixture to give needles, m.p. 153–156° (cap, dec), $[\alpha]_D$ −28° (*c*, 1·4). (Found: C, 74·0; H, 11·2; N, 3·3. $C_{26}H_{47}O_3N$ requires: C, 74·1; H, 11·2; N, 3·3%.)

Reduction of IIId (0·145 g) with LAH (0·5 g) in ether (50 ml) under reflux (5 hr) as described above for IIIa gave the diol (IIIc; 0·10 g) as needles (from benzene–light petroleum) m.p. and mixed m.p. 101–102°.

Oxidation of 15-*hydroxyeperu*-8(20)-*en*-18-*oic acid* (IIId)

The hydroxyacid (0·145 g) in acetone (25 ml) was titrated with the Jones reagent[8] and the solution left with a small excess for 1 hr. MeOH was added, the mixture diluted with water and the product (0·148 g) isolated with ether. Crystallization from aqueous MeOH gave dimorphic needles (0·10 g) of the diacid (IIIa) m.p. and mixed m.p. 150–151° and 158–159°.

Methylation of the α,β-unsaturated γ-lactone acid (Va)

The acid (Va; 2 g) was methylated with diazomethane in ether at 0° and the product chromatographed on neutral alumina (Act III; 100 g). Elution with benzene–light petroleum (1:1) and benzene gave the *methyl ester* (Vb), crystallizing from light petroleum as needles or plates, m.p. 93–94°, $[\alpha]_D$ −58° (*c*, 7·0), λ_{max} = shoulder at ca. 215 mμ (log ε 4·16), end absorption at 200 mμ (log ε 4·31), $\nu_{max}^{CS_2}$ = 3078, 1782, 1749, 1728, 1640, 892, 880 cm^{-1}, $\nu_{max}^{CHCl_3}$ = 1782, 1748, 1719, 1638 cm^{-1}, $\nu_{max}^{CCl_4}$ = 1784, 1754, 1728, 1644 cm^{-1}. (Found: C, 72·8; H, 8·7. $C_{21}H_{30}O_4$ requires: C, 72·8; H, 8·7 %.)

The compound gave a transient deep red colour with alkaline sodium nitroprusside (Legaltest[9]) and an intense violet colour, changing to blue, with *m*-dinitrobenzene in cold ethanolic KOH (Raymond test[10]).

Ozonolysis of polyalthic acid (VIa)

The acid (VIa; 5 g) was dissolved in ethyl acetate (70 ml) and CH_2Cl_2 (200 ml), the solution cooled to −78° and saturated with O_3 (4 hr). The excess O_3 and solvent were removed *in vacuo* and the residue treated with 10% NaOH aq (100 ml) and 30% H_2O_2 (50 ml) portionwise with stirring. After a further 1 hr stirring the solution was left 17 hr at room temp. Acidification and isolation with ether gave a resin (4 g) which was chromatographed on silica gel (150 g). Elution with ether–benzene (1:1) gave the keto-dicarboxylic acid (VIb; 3·0 g) which crystallized from benzene–light petroleum as needles (1·9 g), m.p. 214–216°, $[\alpha]_D$ +19° (*c*, 3·0 EtOH). (Found: C, 65·1; H, 8·2; O, 26·6. Calc. for $C_{16}H_{24}O_5$: C, 64·8; H, 8·2; O, 27·0%.) (Lit.[16] m.p. 211–212° (dec), $[\alpha]_D^{28}$ +11·6° (MeOH.) The IR spectrum (Nujol) was identical with that published.[16]

Ozonolysis of the γ-lactone acid (Va)

The γ-lactone (Va; 0·65 g) was dissolved in CH_2Cl_2 (150 ml) and ozonized (30 min) at −78°. Working up with alkaline peroxide as above gave a resin (0·51 g) which was chromatographed on silicic acid (Act I; 25 g). Elution with ether–benzene (1:3 and 1:1), and recrystallization of the fractions (0·33 g) from benzene–light petroleum gave the ketodiacid (VIb) as needles (0·21 g), m.p. and mixed m.p. 216–218°, $[\alpha]_D$ +24° (*c*, 2·0 EtOH).

Ozonolysis of the γ-lactone ester (Vb)

The ester (Vb; 1·55 g) in ethyl acetate (150 ml) was cooled to −78° and the solution saturated with O_3 (1 hr). Removal of excess O_3 and evaporation of the solvent *in vacuo* gave an oil which was dissolved in acetic acid (10 ml) and ethyl acetate (50 ml) and stirred with Zn dust (5 g) for 2 hr. Excess Zn was removed by filtration, the filtrate diluted with ether and the organic layer washed with $NaHCO_3$ aq, water, dried and the solvent removed *in vacuo*. The neutral product (VIc; 1·1 g) was dissolved in MeOH (55 ml), a solution of $KHCO_3$ (1·5 g) in water (25 ml) added, and the mixture left 18 hr at room temp. Concentration *in vacuo*, dilution with water and isolation of the neutral product with ether gave an oil (0·6 g), which crystallized. $\nu_{max}^{CS_2}$ = 3495, 1725 cm^{-1}. The NMR spectrum ($CDCl_3$) showed it to contain at least 90% of the diketo–alcohol (VId). However repeated crystallization from benzene–light petroleum gave the ketodiol (VII) as needles (0·15 g), m.p. 177–180°, $[\alpha]_D$ −67° (*c*, 0·6 EtOH), $\nu_{max}^{CS_2}$ = 3553, 3457, 1725 cm^{-1}. (Found: C, 66·4; H, 8·7. $C_{18}H_{28}O_5$ requires: C, 66·6; H, 8·7%.)

5,3′,4′-*Trihydroxy*-3,7,8-*trimethoxyflavone* (IXc)

The pigment was regenerated from the triacetate by boiling the latter (1·3 g) in EtOH (250 ml) containing conc H_2SO_4 (6 ml) and water (3 ml) for 2·5 hr. Concentration, and dilution with water afforded yellow needles (0·90 g). Recrystallization from MeOH gave the flavone (IXc) as yellow needles and orange prisms, m.p. 249–250°. (Found: C, 60·1; H, 4·6; CH_3O, 25·7. $C_{18}H_{16}O_8$ requires: C, 60·0; H, 4·5; 3 × CH_3O, 25·8%.) The pigment gave an olive-green ferric chloride test in alcoholic solution and a magenta colour on reduction with Mg and conc HCl aq in EtOH.

Partial methylation of 5,3′,4′-*trihydroxy*-3,7,8-*trimethoxyflavone*

The pigment (1·0 g) in MeOH (250 ml) was treated with excess diazomethane in ether at 0° for 6 hr. The solvent was removed, the product taken up into $CHCl_3$ and the solution washed repeatedly with 2% KOH aq, then water and the solvent evaporated. Crystallization of the KOH-insoluble product (0·92 g) from EtOH gave yellow needles of gossypetin pentamethyl ether (IXa), m.p. and mixed m.p. 161–162°. (lit.[23] m.p. 161–162°.)

Ethylation of 5,3′,4′-*trihydroxy*-3,7,8-*trimethoxyflavone* (IXc)

The pigment (IXc; 1·55 g) in acetone (150 ml) was heated under reflux with K_2CO_3 (8 g) and diethyl sulphate (5 ml) for 25 hr. The solvent was removed *in vacuo* and the residue treated with dil NH_4OH (1:1) for 2 hr. The product (1·8 g) was isolated with $CHCl_3$, dissolved in benzene and filtered through neutral alumina (Act III; 20 g). The eluate (1·45 g) was recrystallized from aqueous MeOH to give the *triethyl ether* as needles, m.p. 155–156°, λ_{max} (log ε) = 254 (4·40), 272 (4·36), 354 (4·37) mμ. (Found: C, 64·9; H, 6·5. $C_{24}H_{28}O_8$ requires: C, 64·9; H, 6·4%.)

Alkaline degradation of 5,3′,4′-*triethoxy*-3,7,8-*trimethoxyflavone*

The triethyl ether of IXc (0·90 g) in EtOH (40 ml) and water (10 ml) was heated under reflux with KOH (10 g) for 8 hr under N_2. The solution was concentrated *in vacuo*, acidified and extracted with ether. Recovery of the acid fraction (0·42 g) with 8% $NaHCO_3$ aq and crystallization from benzene–light petroleum gave needles of 3,4-diethoxybenzoic acid, m.p. and mixed m.p. 167–168°.

5,7,3′,4′-*Tetrahydroxy*-3,8-*dimethoxyflavone* (IXd)

The purified acetate (0·90 g) was hydrolysed by heating in ethanol (50 ml) containing water (1 ml) and conc H_2SO_4 (2 ml) under reflux for 2 hr. Concentration and dilution with water gave the *pigment* (IXd) which crystallized from aqueous MeOH as yellow–orange needles, m.p. 301–303°. (Found: C, 59·0; H, 4·4; CH_3O, 17·6. $C_{17}H_{14}O_8$ requires: C, 59·0; H, 4·1; 2 × CH_3O, 17·9%.) The flavone gave an olive–green ferric chloride test in alcoholic solution and a magenta colour on reduction in EtOH with Mg and conc HCl aq.

The tetraethyl ether of IXd was prepared with excess diethyl sulphate and K_2CO_3 in acetone under reflux (50 hr) as described above for IXc. Crystallization of the product from aqueous MeOH gave needles, m.p. 125–126°, λ_{max} (log ε) = 254 (4·42), 272 (4·40), 354 (4·39) mμ. (Found: C, 65·8; H, 6·8. $C_{25}H_{30}O_8$ requires: C, 65·5; H, 6·6%.)

Alkaline degradation of 5,7,3′,4′-*tetraethoxy*-3,8-*dimethoxyflavone*

The tetraethyl ether of IXd (0·32 g) and KOH (5 g) were heated under reflux with EtOH (25 ml) and water (5 ml) for 8 hr under N_2. The solution was concentrated, acidified and extracted thoroughly with ether. Recovery of the acid fraction (0·14 g) with 8% $NaHCO_3$ aq and crystallization from benzene–light petroleum gave needles of 3,4-diethoxybenzoic acid, m.p. and mixed m.p. 167–168°. Evaporation of the ether mother liquor, and recrystallization of the residue (0·19 g) from light petroleum gave needles of 2-hydroxy-4,6-diethoxy-ω,3-dimethoxyacetophenone (Xa), m.p. and mixed m.p. 123–124°. (lit.[24] m.p. 124–125°.)

Ethylation of the phenol (Xa; 50 mg) with excess diethyl sulphate and K_2CO_3 in acetone under reflux (20 hr) in the usual way and crystallization of the product from light petroleum at −20° gave 2,4,6-triethoxy-ω,3-dimethoxyacetophenone (Xb) as needles, m.p. and mixed m.p. 26–28°. (lit.[24] m.p. 26–28°.)

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