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THE CHEMISTRY OF THE EUPHORBIACEAE—XI[1]

THE DITERPENES OF *RICINOCARPUS MURICATUS* AND THE STEREOCHEMISTRY OF EPERUIC ACID*

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Abstract—The isolation of three new diterpenes from *Ricinocarpus muricatus* Muell. Arg. is described. Their structures have been established as eperuane-8β,15-diol (Ia), eperuane-8β,15,18-triol (VIa) and 15,16-dihydroxyeperu-8(20)-en-18-oic acid (VIIIa). Eperuic acid (IIIb) has been shown to be antipodal to labd-8(20)-en-15-oic acid at all points except C-13.

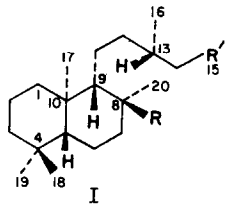
Ricinocarpus stylosus has been shown to contain five new tetracyclic (−)-kauranoid diterpenes[1,2] and the known bicyclic resin acid, polyalthic acid (Xa).[3] In an extension of this work, the diterpenoid constituents of *Ricinocarpus muricatus* Muell. Arg. have been investigated. This plant is a shrub, which is distributed throughout Western Australia in the semi-arid area along the border of the Eremean and South-West provinces. As with many related species, the leaves are coated with a hard resin. Extraction of the milled leaves and terminal branches with ether and washing the extract with sodium bicarbonate solution gave the mono-acid (VIIIa). The neutral extract after saponification yielded the diol (Ia) and the triol (VIa), together with *trans*-cinnamic acid and *p*-methoxy-*trans*-cinnamic acid, indicating that the alcohols may occur as cinnamates as in the case of beyerol.[4,5] The yields of VIIIa, Ia and VIa were 0·23%, 0·28% and 0·04% respectively.

The natural diol (Ia), $C_{20}H_{36}O_2$, had the physical and chemical behaviour of a saturated compound and therefore must be bicarbocyclic. The NMR spectrum of Ia showed the presence of four methyl groups at quaternary positions (9·19(6H), 9·13, 8·85τ) and one methyl group as a doublet (9·09τ; J, 6c/s). A symmetrical two-proton triplet centered at 6·33τ (J, 6c/s) was assigned to the resonance of a $-CH_2 \cdot CH_2 \cdot OH$ grouping. The presence of the primary and tertiary hydroxyl groups in the diol was shown by oxidation of Ia with the Jones reagent[6] to the hydroxy acid (Ib) which was obtained as a crystalline hydrate. Methylation with diazomethane gave the hydroxy-ester (Ic), m.p. 74–75°, $[\alpha]_D$ −3°, the IR spectrum (Nujol) of which

* Part of this work has been described in brief form in Ref. 12.

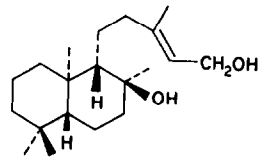
[1] Part X. C. A. Henrick and P. R. Jefferies, *Tetrahedron Letters* No. 24, 1507 (1964).
[2] C. A. Henrick and P. R. Jefferies, *Austr. J. Chem.* **17**, 915 (1964).
[3] K. W. Gopinath, T. R. Govindachari, P. C. Parthasarathy and N. Viswanathan, *Helv. Chim. Acta* **44**, 1040 (1961).
[4] P. R. Jefferies, R. S. Rosich, D. E. White, and M. C. Woods, *Austr. J. Chem.* **15**, 521 (1962).
[5] P. R. Jefferies, R. S. Rosich and D. E. White, *Tetrahedron Letters* No. 26, 1793 (1963).
[6] 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).

was identical with that published[7] for methyl 13-epilabdanolate, m.p. 74–75°, $[\alpha]_D$ +2°, suggesting an enantiomeric relationship. Reduction of Ic with LAH regenerated the diol (Ia).



I

	R	R′
(a)	OH	CH_2OH
(b)	OH	CO_2H
(c)	OH	CO_2Me
(d)	H	CO_2H



II

To confirm this enantiomeric relationship, samples of the enantiomers of methyl labdanolate[8] and 13-epilabdanolate[7,9] were prepared from the diol (II)[10] which has been related to 13-epi-(−)-manoyl oxide.[11] Thus hydrogenation of II over Adam's catalyst followed by oxidation with the Jones reagent[6] and methylation gave a mixture of the 13-epimers, which was separated by chromatography on deactivated alumina as described for the enantiomeric mixture.[9] One of the 13-epimers was identical with methyl *enantio*-13-epilabdanolate (Ic) and the other was identical with methyl *enantio*-labdanolate obtained from *Dodonaea lobulata*.[12]

The ester (Ic) was dehydrated with phosphorus oxychloride in pyridine[8] at 0° to give the expected mixture of double bond isomers.[13,14] The NMR spectrum of the product showed it to contain about 70% of the exocyclic isomer (IIIa) and 20% of the 7(8)-trisubstituted isomer, the remainder presumably being due to the tetrasubstituted 8(9)-isomer. Chromatography of the mixture on silica gel impregnated with silver nitrate[15] enabled the separation of the pure exocyclic isomer (IIIa), which was hydrolysed to the acid (IIIb), characterized as the cyclohexylamine salt. Ozonolysis of IIIa and saponification of the product gave the keto-acid (IIIc) characterized as its oxime, m.p. 224–226°, $[\alpha]_D$ −86°. This proved to be identical to the corresponding oxime derived from eperuic acid[16] and thus establishes eperuic acid as IIIb with a skeleton antipodal to labd-8(20)-en-15-oic acid[8] at all points except C-13.[12]*

* E. M. Graham and K. H. Overton have arrived at the same conclusion concerning the relationship of labdanolic and eperuic acids. (Personal communication). cf. *J. Chem. Soc.* 126 (1965).

[7] S. Bory and E. Lederer, *Croat. Chem. Acta* **29**, 157 (1957).

[8] J. D. Cocker and T. G. Halsall, *J. Chem. Soc.* 4262 (1956); *ibid.* 4401 (1957).

[9] D. B. Bigley, N. A. J. Rogers and J. A. Barltrop, *J. Chem. Soc.* 4613 (1960).

[10] P. R. Jefferies and T. G. Payne, *Austr. J. Chem.* **18**, in the press.

[11] B. E. Cross, R. H. B. Galt, J. R. Hanson, P. J. Curtis, J. F. Grove and A. Morrison, *J. Chem. Soc.* 2937 (1963).

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

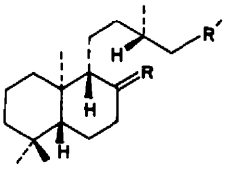
[13] T. Nakano and C. Djerassi, *J. Org. Chem.* **26**, 167 (1961).

[14] R. Hodges and R. I. Reed, *Tetrahedron* **10**, 71 (1960).

[15] T. Norin and L. Westfelt, *Acta Chem. Scand.* **17**, 1828 (1963).

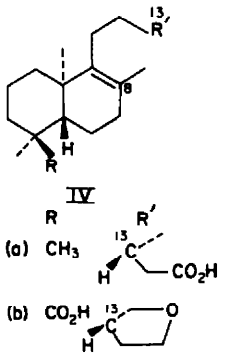
[16a] F. E. King and G. Jones, *J. Chem. Soc.* 658 (1955);

[16b] S. Blake and G. Jones, *Ibid.* 430 (1963).



III

	R	R′
(a)	CH_2	CO_2Me
(b)	CH_2	CO_2H
(c)	O	CO_2H
(d)	CH_2	CH_2OH
(e)	CH_2	CH_3

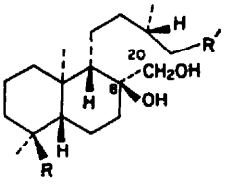


IV

	R	R′
(a)	CH_3	^{13}C H CO_2H
(b)	CO_2H	^{13}C H O

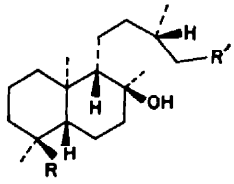
Isomerization of IIIa was effected with sulphuric acid in refluxing methanol to give, after saponification, the crystalline acid (IVa). In the NMR spectrum of IVa the 8-methyl group absorbed as a singlet at 8·44τ. Hydrogenation of IVa over Adams' catalyst gave dihydroeperuic acid (Id) which was obtained crystalline and converted to the known *p*-bromophenacyl ester.[16a] The change in molecular rotation (+183°) which occurred on hydrogenation of IVa is consistent with that observed for analogous labdanolic[8] and grindelic[17] acid derivatives. The 8α-methyl in Id is formulated on the assumption that hydrogenation occurred at the less hindered β-face of the molecule.* Reduction of methyl eperuate (IIIa) with LAH gave the alcohol (IIId) which was converted to the triol (Va) with osmium tetroxide in pyridine.[18] The toluene-*p*-sulphonate of IIId was reduced smoothly with LAH in tetrahydrofuran to give eperu-8(20)-ene (IIIe), which was characterized by conversion with osmium tetroxide in pyridine to the crystalline diol (Vb). In the NMR spectrum of Vb the methylene hydrogens of the 8-hydroxymethyl group absorbed as an almost equivalent AB system centred at 6·44τ (J, 11·5 c/s).

The IR spectra in dilute solution (CCl_4) of the diol (Ia) and the ester (Ic) in the



V

	R	R′
(a)	CH_3	CH_2OH
(b)	CH_3	CH_3
(c)	CH_2OTs	CH_3



VI

	R	R′
(a)	CH_2OH	CH_2OH
(b)	CH_2OAc	CH_2OAc
(c)	CHO	CO_2H

* In all cases of addition to olefinic centres at C-8, attack by the reagent is formulated as proceeding from the less hindered β-face of the double bond.[8,17,19]

[17] L. Panizzi, L. Mangoni and M. Berlardini, *Gazz. Chim. Ital.* **92**, 522 (1962).
[18] J. S. Baran, *J. Org. Chem.* **25**, 257 (1960).
[19] L. Mangoni and M. Belardini, *Gazz. Chim. Ital.* **93**, 465 (1963).

region 3000–3700 cm^{-1} are very similar to these reported[20] for the corresponding labdanolic acid derivatives. Thus, both 13-epimers exhibit intramolecular hydrogen bonding in dilute solutions of carbon tetrachloride.*

The natural triol (VIa), $C_{20}H_{38}O_3$, on mild acetylation gave an oily diacetate (VIb), hydrolysis of which regenerated the triol. The NMR spectrum of VIa showed the presence of three methyl groups at quaternary positions (Table 2), and a split methyl group (doublet at 9·08τ; J, 6 c/s). An AB quartet centred at 6·78τ (J, 11 c/s) is assigned to the resonance of the C-18 hydrogens, and a two-proton triplet centred at 6·35τ (J, 6 c/s) is attributable to the resonance of the C-15 hydrogens. In the spectrum of the triol diacetate (VIb) the C-15 hydrogens absorbed as a triplet centred at 5·88τ (J, 6 c/s) and the methylene hydrogens of the 4-acetoxymethyl group absorbed as an AB quartet centred at 6·24τ (J, 10·5 c/s). Oxidation of the triol (VIa) with six equivalents of the Jones reagent[8] gave the oxo-acid (VIc), characterized as its oxime. Wolff-Kishner reduction of VIc, followed by methylation, gave the hydroxy-ester (Ic), identical with that obtained from the natural diol (Ia). In the NMR spectrum of the oxoacid (VIc), the 4-equatorial aldehyde proton absorbed as a singlet at 0·78τ. The average chemical shifts of the C-18 protons in VIa, VIb and VIc are in close agreement with values for analogous diterpenoids with equatorial 4-substituents,[2,21] and so the stereochemistry of the triol is expressed as VIa.

Dehydration of the triol diacetate (VIb) with phosphorous oxychloride in pyridine again gave a mixture of double bond isomers containing ca. 68% of the exocyclic olefin as determined by the NMR spectrum of the mixture.† Saponification of the mixture and repeated crystallization of the diols enabled the separation of the pure exocyclic isomer (VIIa).

The original assignment[7,9] to the 13-configuration in labdanolic acid is taken to be correct, since the intramolecular hydrogen-bonding observed for methyl labdanolate[20] and Ic does not appear to invalidate this assignment.[22] The molecular rotation differences between the corresponding derivatives of eperuic and *enantio*-labdanolic acids are listed in Table 1. The validity of comparing the $[M]_D$ difference between methyl labdanolate and 13-epilabdanolate with those of enantiomeric 3-methyl fatty acids has been criticized on the grounds of hydrogen bonding in the former.[20] Table 1 includes a number of $\Delta[M]_D$ values involving compounds in which hydrogen bonding is unlikely to be significant. In particular, the molecular rotation difference (+15°) between Id and *enantio*-dihydrocativic acid[23] is in agreement with values for enantiomeric 3-methyl fatty acids.[7,24]

The IR spectrum (Nujol) of the dihydroxy-acid (VIIIa), $C_{20}H_{34}O_4$, showed

* cf. Ref. 9.

† cf. Ref. 13.

[20] A. J. Baker, G. Eglinton, A. G. Gonzalez, R. J. Hamilton and R. A. Raphael, *J. Chem. Soc.* 4705 (1962).

[21a] E. Wenkert and P. Beak, *Tetrahedron Letters* No. 11, 358 (1961); A. Gaudemer, J. Polonsky and E. Wenkert, *Bull. Soc. Chim. Fr.* 407 (1964);

[b] W. R. Chan, C. Willis, M. P. Cava and R. P. Stein, *Chem. & Ind.* 495 (1963);

[c] R. A. Laidlaw and J. W. W. Morgan, *J. Chem. Soc.* 644 (1963).

[22] L. Mangoni and M. Belardini, *Gazz. Chim. Ital.* **92**, 1379 (1962).

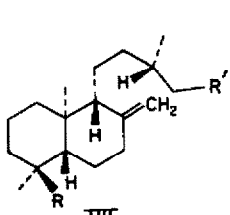
[23] H. H. Zeiss and F. W. Grant, Jr., *J. Amer. Chem. Soc.* **79**, 1201 (1957).

[24] S. Ställberg-Stenhagen, *Arkiv. Kemi* **26A**, No. 1 (1948); *Ibid.* **26A**, No. 12 (1948); *Chem. Abstr.* **43**, 6160c (1949).

TABLE 1.—MOLECULAR ROTATION DIFFERENCES BETWEEN DERIVATIVES OF EPERUIC AND ENANTIO-LABDANOLIC* ACIDS

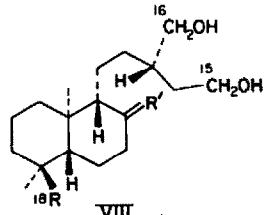
Eperuic acid compound →	*enantio*-Labdanolic acid compound*	$\Delta[M]_D$
Ia	Labdane-8α,15-diol[20]	+19°
Ib	Labdanolic acid[6]	+42°
Ic	Methyl labdanolate[6]	+37°
Va	Labdane-8α,15,20-triol[20]	+43°
IIIa	Methyl labd-8(20)-en-15-oate[6]	+29°
cyclohexylamine salt of IIIb	Salt of labd-8(20)-en-15-oic acid[6]	+40°
IIIc	20-Nor-8-oxo-labdan-15-oic acid[6]	+61°
oxime of IIIc	Oxime of 20-nor-8-oxo-labdan-15-oic acid[6]	+37°
IVa	Labd-8-en-15-oic acid[6]	+65°
Id	Dihydrocativic acid[23]	+15°

* The sign of rotation of all labdanolic acid compounds is reversed to correspond to the *enantio*-series.



VII

	R	R'
(a)	CH_2OH	CH_2OH
(b)	CH_2OTs	CH_3
(c)	CH_2OAc	CH_2OAc



VIII

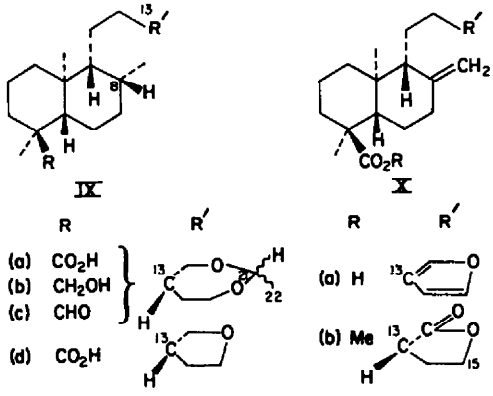
	R	R'
(a)	CO_2H	CH_2
(b)	CO_2Me	CH_2
(c)	CO_2H	Me, H
(d)	CH_2OH	CH_2
(e)	CH_2OH	Me, H

absorption at 3085, 1645 and 885 cm^{-1} attributed to a vinylidene group. Methylation with diazomethane afforded the methyl ester (VIIIb) and acetylation of the acid under mild conditions gave an oily diacetate characterized as its cyclohexylamine salt. The NMR spectra of the methyl ester (VIIIb) and the diacetate of VIIIa both showed only two skeletal methyl signals as singlets. The chemical shifts of the methyl groups (Table 2), when compared with the shifts observed for polyalthic acid (Xa) derivatives,[2] indicated an equatorial 4-carboxyl group in the natural acid (VIIIa). The spectra showed the vinylidene proton signals as broad singlets at 5·18 (IH) and 5·48τ (IH). The 15- and 16-hydrogen signals in the spectrum of the ester (VIIIb) were present as a broad multiplet (4H) centred at 6·37τ, shifted downfield to 5·87τ in the spectrum of the diacetate of VIIIa.

The IR spectrum (CCl_4; 0·004 M) of the methyl ester (VIIIb) showed free hydroxyl absorption at 3634 cm^{-1} and intramolecularly hydrogen bonded hydroxyl absorption at 3470 cm^{-1}, values which correspond closely to those (3636, 3477 cm^{-1}) of butane-1,4-diol.[25]

[25] L. P. Kuhn, P. von R. Schleyer, W. F. Baitinger, Jr., and L. Eberson, *J. Amer. Chem. Soc.* **86**, 650 (1964).

Hydrogenation of VIIIa gave a mixture from which the major isomer (VIIIc) was readily separable. The acids (VIIIa and VIIIc) had pK^{*}_{MCS} values[26] of 7·83 and 7·98 respectively, consistent with an equatorial 4-carboxyl group (calc. 7·91).[26] Reduction of the acid (VIIIa) or the methyl ester (VIIIb) with LAH gave the triol (VIIId). Hydrogenation of the triol again gave a mixture from which the 8α-methyl isomer (VIIIe) was separated.



The diol (VIIIc) and the triol (VIIIe) were easily converted into the corresponding ethylidene derivatives (IXa and IXb). The NMR spectrum of the latter showed the ethylidene 21-H signal as a quartet at 5·11τ (J, 5·1 c/s) and the ethylidene methyl signal as a doublet at 8·75τ (J, 5·1 c/s). The alcohol (IXb) was oxidized with chromic acid–pyridine[27] to the corresponding aldehyde (IXc). The average chemical shifts of the C-18 protons in the triacetate of VIIId, AB quartet at 6·24τ (J, 10·6 c/s); the ethylidene derivative (IXb), AB quartet at 6·77τ (J, 11·0 c/s;) and the aldehyde (IXc), singlet at 0·80τ, support an equatorial orientation of the functional groups at C-4.[2,21]

Other evidence indicating the proximity of the two primary alcohol groups was obtained by dehydration of the diol (VIIIc) with *p*-toluene sulphonic acid in refluxing benzene. A high yield of the crystalline cyclic ether (IXd) was obtained, the IR (CS_2) and NMR spectra of which were indistinguishable from those of the hydrogenation product of polyalthic acid (Xa). However, hexahydropolyalthic acid could not be obtained crystalline and is no doubt a mixture of epimers at C-8 and at C-13.

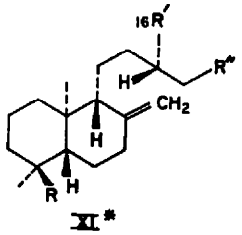
Treatment of the ester (VIIIb) with sulphuric acid in methanol brought about isomerization of the exocyclic double bond to the tetrasubstituted position and simultaneous dehydration of the 1,4-diol system to afford a tetrahydrofuran, which after saponification gave the acid (IVb), which was hydrogenated to IXd. The change in molecular rotation (+118°) which occurred on hydrogenation of IVb to IXd agrees with that recorded for analogous systems.[8,17] The production of IXd on hydrogenation of IVb proves that no skeletal rearrangement occurred during the acid isomerization and that the configuration of the side chain at C-9 must be α (equatorial).[8]

[26] 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).

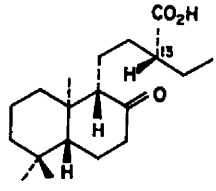
[27] G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *J. Amer. Chem. Soc.* **75**, 422 (1953).

Oxidation of the diol (VIIIb) with the Jones reagent[6] gave a neutral product which contained negligible aldehyde and appeared to be a mixture of the two possible γ-lactones.[28] One isomer (Xb) was separated from this mixture by chromatography and fractional crystallization. The IR spectrum (CS_2) showed carbonyl absorption at 1732 (ester) and 1783 (γ-lactone) cm^{-1}, and the formation of a γ-lactone on oxidation proves a 1,4-relationship between the hydroxyl groups in the natural acid (VIIIa). The assignment of the structure Xb to this lactone is based on its subsequent degradation. In the NMR spectrum of Xb the C-15 hydrogens absorbed as a multiplet (2H) centred at 5·70τ. Reduction of the γ-lactone mixture with LAH gave the triol (VIIId).

To establish the stereochemistry of VIIIa by conversion to eperu-8(20)-ene (IIIe), tosylation of the triol (VIIId) was examined. Although 1,4-diols are converted partly to tetrahydrofurans with tosyl chloride in pyridine,[29] under modified conditions, smooth conversion to the tritosylate occurred. Reduction of this tritosylate with LAH in ether gave a mixture from which the hydrocarbon (IIIe) and the mono-toluene-*p*-sulphonate (VIIb) were separated. Treatment of IIIe with osmium tetroxide in pyridine gave the diol (Vb) identical with the sample prepared from methyl eperuate (IIIa). Similar hydroxylation of VIIb gave the diol (Vc), the NMR spectrum of which showed the resonance of the C-18 hydrogens as an AB quartet centred at 6·38τ (J, 9 c/s). By comparison with the chemical shifts quoted for axial 4-CH_2OTs groups (ca. 6·05τ),[2] it can be seen that the NMR spectra of tosylates can also be used to indicate the C-4 orientation.



XI*



XII*

	R	R′	R″
(a)	CO_2H	CO_2H	CH_2SCH_2Ph
(b)	CO_2Me	CO_2Me	CH_3
(c)	CH_2OH	CH_2OH	CH_3
(d)	CHO	CO_2H	CH_3

Degradation of the γ-lactone (Xb) was brought about by alkyl oxygen fission with sodium benzylmercaptide[30] in dimethylformamide[2] to give the benzylthioether (XIa) in which hydrolysis of the 4-ester function had also occurred. The thioether was methylated directly and desulphurized with deactivated Raney nickel yielding XIb. In the NMR spectrum of the latter, the carbomethoxy methyl groups absorbed as singlets at 6·30 and 6·33τ and the C-15 hydrogens absorbed as an unsymmetrical

* Some epimerisation of the C-13 stereochemistry may have occurred under the alkaline degradation conditions.

[28] V. I. Stenberg and R. J. Perkins, *J. Org. Chem.* **28**, 323 (1963).
[29] G. A. Haggis and L. N. Owen, *J. Chem. Soc.* 389 (1953).
[30] cf. J. W. Reppe, *Liebigs Ann.* **596**, 170 (1955).

triplet centred at 9·12τ (J, 6·5 c/s). The mass spectrum of the dimethyl ester (XIb) was virtually indistinguishable from that quoted for dimethyl pinifolate (XIII).[31] Reduction of XIb with LAH gave the diol (XIc), which was oxidized with six equivalents of the Jones reagent[6] to give the aldehyde XId. Wolff-Kishner reduction of the latter and ozonolysis of the product gave XII characterized as its oxime.

TABLE 2. CHEMICAL SHIFTS OF METHYL GROUPS IN EPERUANE DERIVATIVES
(τ values[32] in $CHCl_3$ or $CDCl_3$)

Compound	Equatorial C-4 Group	C-8 Group	C-4 Methyl	C-10 Methyl	C-20 Hydrogens
IIIa	CH_3	8(20)-ene	9·12, 9·19	9·31	5·18, 5·50
VIIa	CH_2OH	8(20)-ene	9·24	9·27	5·18, 5·48
VIIc	CH_2OAc	8(20)-ene	9·17	9·28	5·19, 5·50
VIIb	CH_2OTs	8(20)-ene	9·24	9·32	5·20, 5·47
Tritosylate of VIIId	CH_2OTs	8(20)-ene	9·27	9·40	5·24, 5·65
Diacetate of VIIIa	CO_2H	8(20)-ene	8·84	9·28	5·14, 5·48
VIIIb	CO_2Me	8(20)-ene	8·86	9·30	5·18, 5·48
IIIc	CH_3	8C=O	9·02, 9·14	9·28	—
IVa	CH_3	8-ene	9·11, 9·16	9·05	8·44
IVb	CO_2H	8-ene	8·79	9·01	8·42
Id	CH_3	8αCH_3, 8βH	9·14, 9·17	9·14	9·12(d)
IXb	CH_2OH	8αCH_3, 8βH	9·25	9·13	9·12(d)
IXa	CO_2H	8αCH_3, 8βH	8·83	9·13	9·12(d)
IXc	CHO	8αCH_3, 8βH	8·94	9·10	9·11(d)
Ic	CH_3	8αCH_3, 8βOH	9·13, 9·19	9·19	8·85
VIa	CH_2OH	8αCH_3, 8βOH	9·25	9·16	8·85
VIb	CH_2OAc	8αCH_3, 8βOH	9·18	9·16	8·84
VIc	CHO	8αCH_3, 8βOH	8·95	9·14	8·82
Vb	CH_3	8αCH_2OH, 8βOH	9·12, 9·20	9·26	6·44*
Vc	CH_2OTs	8αCH_2OH, 8βOH	9·26	9·26	6·48*

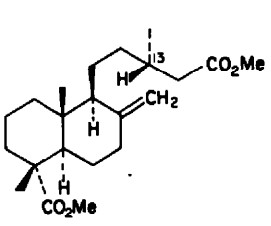
(d) = doublet (J, 7 c/s)
* AB (Almost A_2) system (J, 11·5 c/s)

The chemical shifts (τ-values[32]) of the methyl groups in the various eperuane derivatives with variation of the C-8 and C-4 substituents are listed in Table 2. In the spectra of compounds containing a 4-*gem*-dimethyl grouping, the line positions of the 4-methyl groups (ca. 9·13, 9·19) are essentially the same as those in 16α-(−)-kaurane, beyerane and phyllocladane.[2,5] The effects on the C-4 and the C-10 methyl line positions of varying the 4-equatorial substituent are considerably different from those observed in analogous compounds containing 4-axial substituents.[2] Thus the 10-methyl resonance is practically unaffected by variation of the 4-equatorial function in contrast to the analogous 4-axial series.[2] In addition, a 4-axial CH_2OH group deshields the 4-(eq) methyl group,[2] whereas in the alternative situation the 4-axial methyl group is slightly shielded, effects which probably originate from conformational differences. Generally, these chemical shift differences serve to distinguish axial and equatorial oxygenation at the 4-position. In this connection, the assignment by

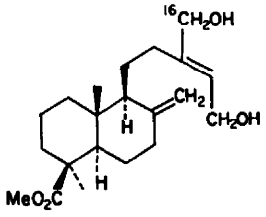
[31] C. Enzell and O. Theander, *Acta Chem. Scand.* **16**, 607 (1962).
[32] G. V. D. Tiers, *J. Phys. Chem.* **62**, 1151 (1958).

Rowe and Scroggins of an equatorial 4-hydroxymethyl group to a hydroxy-13-epimanool[33] does not agree with the NMR data quoted, which is consistent with an axial 4-CH_2OH group.[2]

The deshielding of the 10-methyl group by the introduction of the axial 8α-methyl on hydrogenation of the vinylidene group has been considered before.[2] The removal of the anisotropic vinylidene group on hydrogenation could account for part (ca. 0·05 ppm) of this downfield shift of the 10-methyl signal.* On replacing the vinylidene group by an 8-ketone function, the 10-methyl chemical shift is practically unchanged, whereas at least one of the 4-methyl groups is deshielded. The 8-ene function deshields the 10-methyl group but has little effect on the 4-methyl groups. Whereas a 4-axial CH_2OH group has the same effect on the 10-methyl as does a 4-axial CH_3 group,[2] the hydrogen bonded 8β,20-diol grouping in Vb has an overall shielding effect on the 10-methyl relative to the effect of an 8α-methyl group. In the tritosylate of VIIId the 15- and/or 16-tosylate groups shield both the 10-methyl group and the 20-hydrogens.



XIII



XIV

The bicyclic constituents of *R. muricatus*, together with eperuic acid, represent the only compounds described to date with the stereochemistry of eperu-8(20)-ene (IIIe). Compounds which are largely enantiomeric to the diol (Ia) and the triol (VIa), namely labdane-8α,15-diol and labdane-8α,15,18-triol have been found recently to co-occur.[35] Oxygenation at C-16 in the acid (VIIIa) represents a link between the diterpenes of *R. muricatus* and the occurrence of polyalthic acid (Xa) in *R. stylosus*.[2] Other examples of 16-oxygenation other than β-substituted furans, are methyl sciadopate (XIV)[36]† and andrographolide.[37]

EXPERIMENTAL

Analyses were carried out by the Australian Microanalytical Service, Melbourne. Rotations were determined in $CHCl_3$ unless otherwise stated, and at room temp (19–24°). All identities were confirmed by comparison of IR spectra measured with Perkin-Elmer infracords 137 and 137G for the ranges

* cf. Ref. 34.

† M. Sumimoto *et al.*[36] assign a *cis*-relationship between the two hydroxymethyl groups in methyl sciadopate. However, they obtained a diacid on Jones oxidation of XIV, and in view of the formation of γ-lactones on oxidation of VIIIb and especially of *cis*-1,4-butenediol,[38] the *trans*-structure (XIV) appears more likely.

[33] J. W. Rowe and J. H. Scroggins, *J. Org. Chem.* **29**, 1554 (1964).

[34] W. A. Ayer, C. E. McDonald and J. B. Stothers, *Canad. J. Chem.* **41**, 1113 (1963).

[35] C. Tabacik-Wlotzka, M. Mousseron and A. Chafai, *Bull. Soc. Chim. Fr.* 2299 (1963).

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

[37] M. P. Cava, B. Weinstein, W. R. Chan, L. J. Haynes and L. F. Johnson, *Chem. & Ind.* 167 (1963); see also Ref. 21*b*.

1300–670 cm^{-1} and 4000–1300 cm^{-1} respectively. More precise results were determined in CCl_4 solution using 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.[22] 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. The mass spectrum was recorded on an Atlas CH4 instrument with a heated all glass inlet system at 200°. The energy of the electrons was 70 eV.

Isolation of the diterpenoids of Ricinocarpus muricatus

Dried crushed plant (13 kg) collected 15 miles south of Norseman, Western Australia, in October 1961 was extracted with cold ether. The extract was washed thoroughly with 8% $NaHCO_3$aq and then 5% NaOHaq. Isolation of the acids from the former solution with 5% HClaq and ether gave a residue which soon crystallized. The acid (30 g) was obtained by washing with a little ether and recrystallized from acetone to afford plates of 15,16-*dihydroxyeperu*-8(20)-*en*-18-*oic acid* (VIIIa), m.p. 152–153°, $[\alpha]_D$ −38° (*c*, 3·0 EtOH), ν_{max}^{Nujol} = 3490, 3230, 3085, 1685, 1645, 1235, 885 cm^{-1}, pK_{MCS}^{*} 7·83, equiv. wt. 345 (Found: C, 70·9; H, 9·9. $C_{20}H_{34}O_4$ (338·5) requires: C, 71·0; H, 10·1%.) The neutral extract was saponified with 5% KOH in methanol under reflux. The methanol was removed under red. press., water added and the mixture extracted with ether to afford a neutral fatty oil, which was dissolved in MeOH–light petroleum (1:1; 1 l). Water (65 ml) was added with shaking and the aqueous MeOH layer separated, washed with light petroleum (500 ml), dried (Na_2SO_4) and the solvent removed *in vacuo* to give a red oil (45 g), which was chromatographed on neutral alumina (Act III; 1 kg). Elution with benzene–light petroleum (1:1), benzene, benzene–ether mixtures and ether gave *eperuane*-8β,15-*diol* (Ia; 36 g), which crystallized from light petroleum at −15° as needles, m.p. 75–76°, $[\alpha]_D$ +4° (*c*, 9), $\nu_{max}^{CCl_4}$ (0·0045 M) = 3632, 3610 sh, 3505, 3370 cm^{-1}. (Found: C, 77·6; H, 12·2; O, 10·2. $C_{20}H_{36}O_2$ requires: C, 77·4; H, 12·3; O, 10·3%.) Elution with ether–MeOH (1:1) and MeOH gave an oil (9 g) which slowly crystallized. Washing with a little ether and recrystallization of the insoluble portion from benzene afforded *eperuane*-8β,15,18-*triol* (VIa; 5 g) as needles, m.p. 121–122°, $[\alpha]_D$ −13° (*c*, 8·2, EtOH) (Found: C, 73·6; H, 11·5; O, 14·7. $C_{20}H_{38}O_3$ requires: C, 73·6; H, 11·7; O, 14·7%.)

The aqueous KOH mother liquor from the above saponification of the neutral extract was acidified and extracted with ether. The acids were removed from the organic layer with 8% $NaHCO_3$ aq, the washings re-acidified and extracted with ether to give a crystalline acid fraction (30 g). Chromatography on activated charcoal (200 g) and elution with ether gave *trans*-cinnamic acid (15 g), as needles (from water), m.p. and mixed m.p. 133–134°. Elution with ether and ether–MeOH (1:1) gave *p*-methoxy-*trans*-cinnamic acid (7 g), as needles (from aqueous MeOH), m.p. and mixed m.p. 172–173°.

Chromic acid oxidation of eperuane-8β,15-*diol* (Ia)

The diol (Ia; 2 g) in acetone (25 ml) was titrated with the Jones reagent[6] and the solution left with a small excess for 15 min. MeOH was added, the mixture diluted with water and extracted with ether. The organic layer was washed with 5% Na_2CO_3aq, the washings acidified and extracted with ether to give 8β-*hydroxy*-*eperuan*-15-*oic acid* (Ib; 1·8 g). Crystallization from aqueous MeOH gave solvated needles, m.p. 104–108° (cap), $[\alpha]_D$ −6° (*c*, 4·5, $CHCl_3$–EtOH; 1:1) (Found: C, 70·1; H, 11·2. $C_{20}H_{36}O_2 \cdot H_2O$ requires: C, 70·1; H, 11·2%). On drying *in vacuo* at 60° the crystalline hydrate became a resin (Found: C, 73·6; H, 11·0. $C_{20}H_{36}O_2$ requires: C, 74·0; H, 11·2%.)

Methylation with excess diazomethane in ether gave *methyl*-8β-*hydroxyeperuan*-15-*oate* (Ic), crystallizing from light petroleum at −15° as needles, m.p. 74–75°, $[\alpha]_D$ −3° (*c*, 11·0), $\nu_{max}^{CCl_4}$ (0·006 M) = 3609, 3545 cm^{-1}. (Found: C, 74·7; H, 11·1. $C_{21}H_{38}O_2$ requires: C, 74·5; H, 11·3%). The IR spectrum (Nujol) was identical with that quoted[7] for methyl 13-epilabdanolate. Reduction of the ester (Ic) with LAH in ether regenerated the diol (Ia) as needles (from light petroleum at −15°), m.p. and mixed m.p. 75–76°, $[\alpha]_D$ +4° (*c*, 12·0).

Preparation of methyl enantio-13-*epilabdanolate and methyl* enantio-*labdanolate*

Eperu-13-en-8β,15-diol[10] (II; 7·0 g) in MeOH (100 ml) was shaken with H_2 at room temp and press in the presence of PtO_2 (1·0 g). One mole H_2 was rapidly absorbed and after 1·5 hr the catalyst

was removed and the solvent evaporated *in vacuo*. The mixture of diols was dissolved in acetone (100 ml) and treated with a small excess Jones reagent.[6] After 30 min, MeOH (5 ml) was added, the mixture diluted with water and extracted with ether. Recovery of the acids (6·0 g) with 5% Na_2CO_3aq and methylation with diazomethane in ether afforded a crystalline mixture of esters which was chromatographed on neutral alumina (Act IV; 400 g). Elution with benzene–light petroleum and with benzene gave methyl *enantio*-13-epilabdanolate (2·65 g) which, after 3 recrystallizations from light petroleum at −15°, formed needles (0·75 g), m.p. 73–74°, undepressed on admixture with methyl 8β-hydroxy-eperuan-15-oate (Ic). Further elution with benzene gave mixtures (0·95 g) of the two esters. Elution with benzene–ether (1:1) gave methyl *enantio*-labdanolate (2·60 g) which was recrystallized (3X) from light petroleum at −15° to give needles (0·65 g), m.p. and mixed m.p. 72–73°. The mixed m.p. with Ic was 56–58°. (lit.[12] m.p. 72–74°). The IR spectrum (Nujol) was identical with that published[7] for methyl labdanolate.

Dehydration of methyl 8β-hydroxy-eperuan-15-oate (Ic)

The alcohol (Ic; 18·80 g) in pyridine (200 ml) was cooled in an ice bath and a mixture of $POCl_3$ (50 ml) and pyridine (100 ml) was slowly added. After 18 hr at 0° the mixture was poured slowly, with stirring, into ice–$NaHCO_3$aq. Isolation of the neutral product with ether gave an oil (17·5 g) which was chromatographed on neutral alumina (Act III; 100 g). Elution with light petroleum gave a colourless oil (16·9 g), the NMR spectrum of which showed it to be a mixture containing ca. 70% of the 8(20)-olefin, 20% of the 7(8)-olefin and 10% of the 8(9)-isomer. The olefin mixture (13·5 g) was chromatographed on $AgNO_3$-silica gel[15] (400 g). Elution with ether-light petroleum (1:24 and 1:19) gave mixtures (9·60 g) of the olefin isomers. Elution with ether-light petroleum (1:19, 1:9 and 1:1) gave *methyl eperu-8(20)-en-15-oate* (IIIa; 3·28 g), b.p. 168°/1 mm, $[\alpha]_D$ −36° (*c*, 5·9) (Found: C, 78·7; H, 11·2. Calc. for $C_{21}H_{36}O_2$: C, 78·7; H, 11·3%.) (lit.[16] for methyl eperuate, b.p. 164°/0·4 mm, $[\alpha]_D^{18}$ −28·2°). The olefin mixture (9·60 g) above was rechromatographed on $AgNO_3$–silica gel (400 g). Elution with ether–light petroleum (1:24 and 1:19) again gave mixtures (6·70 g) whereas elution with ether–light petroleum (1:9 and 1:1) gave a further quantity of pure IIIa (2·20 g).

Hydrolysis of IIIa with 10% KOH in MeOH (3 hr reflux) gave IIIb as a gum which could not be crystallized. The *cyclohexylamine salt*, prepared in hot ethyl acetate, was recrystallized from the same solvent as needles, m.p. 133–136° (cap, dec), $[\alpha]_D$ −32° (*c*, 5·6) (Found: C, 77·1; H, 11·6; N, 3·7. Calc. for $C_{26}H_{47}O_2N$: C, 77·0; H, 11·7; N, 3·5%.) (lit.[16b] m.p. 128–132°, $[\alpha]_D$ −30·2°).

Ozonolysis of methyl eperu-8(20)-en-15-oate (IIIa)

The olefin (IIIa; 1·0 g) in methyl acetate (40 ml) was cooled to −70° and the solution saturated with ozone. The excess ozone was removed, the solvent evaporated *in vacuo* and the residue heated with water (50 ml) on a steam bath for 2 hr. The neutral product was isolated with ether, dissolved in light petroleum and filtered through neutral alumina (Act IV; 30 g) to give the keto-ester (0·75 g), which was hydrolysed with 10% KOH in MeOH under reflux for 2 hr to afford *20-nor-8-oxo-eperuan-15-oic acid* (IIIc) as a gum, $[\alpha]_D$ +20° (*c*, 6·7). The keto-acid was characterized as the *oxime*, which crystallized from MeOH as needles, m.p. 224–226° (dec.), $[\alpha]_D$ −86° (*c*, 1·0, dioxan), −85° (*c*, 2·9, pyridine). (Found: C, 70·4; H, 10·3. Calc. for $C_{19}H_{33}O_3N$: C, 70·6; H, 10·3%.) The m.p. was undepressed on admixture with an authentic sample derived from methyl eperuate (lit.[16a] m.p. 223°, $[\alpha]_D^{20}$ −79·4° (dioxane);* m.p. 224–226°, $[\alpha]_D$ − 87° (pyridine).).

Isomerization of methyl eperu-8(20)-en-15-oate (IIIa)

The olefin mixture (5·50 g), from the $POCl_3$ dehydration, was dissolved in MeOH (250 ml) containing conc. H_2SO_4 (20 ml) and the solution heated under reflux for 5 hr. Dilution with water, isolation of the neutral product with ether and filtration through neutral alumina (Act IV; 100 g) in light petroleum gave methyl eperu-8-en-15-oate (5·40 g) as a mobile oil. Hydrolysis with 10% KOH in MeOH (2 hr reflux) afforded *eperu-8-en-15-oic acid* (IVa), which crystallized from aqueous MeOH as needles, m.p. 70–71°, $[\alpha]_D$ −90° (*c*, 4·0). (Found: C, 78·4; H, 11·3. $C_{20}H_{34}O_2$ requires: C, 78·4; H, 11·2%.)

* K. H. Overton, personal communication.

Hydrogenation of eperu-8-en-15-oic acid (IVa)

The acid (IVa; 3·5 g) in acetic acid (75 ml) was hydrogenated at room temp and press in the presence of Adams' catalyst (0·90 g). The uptake of H_2 ceased after 30 min. After removal of the catalyst, dilution with water and isolation with ether gave 8β(H)-*eperuan-15-oic acid* (Id) which crystallized from aqueous MeOH as needles, m.p. 62–63° $[\alpha]_D$ −30° (*c*, 2·6) (Found: C, 77·8; H, 11·6. $C_{30}H_{50}O_2$ requires: C, 77·9; H, 11·8%). The p-*bromophenacyl ester* crystallized from aqueous MeOH as needles, m.p. 72–73°, $[\alpha]_D$ −28° (*c*, 4·5). (Found: C, 66·4; H, 8·2; Br, 15·9. Calc. for $C_{38}H_{51}O_3Br$: C, 66·5; H, 8·2; Br, 15·8%.) (Lit.[16a] m.p. 71–71·5°).

The *methyl ester*, prepared by heating the acid (1·7 g) in MeOH (50 ml) containing conc H_2SO_4 (1·5 ml) under reflux for 3 hr, was a mobile oil, b.p. 135°/0·1 mm, $[\alpha]_D$ −25° (*c*, 10·0). (Found: C, 78·2; H, 11·7. Calc. for $C_{31}H_{50}O_2$: C, 78·2; H, 11·9%.) (Lit.[16a] b.p. 138–140°/0·1 mm, $[\alpha]_D^{25}$ −26·3°.)

Reduction of methyl eperu-8(20)-en-15-oate (IIIa)

The ester (IIIa; 2·50 g) in ether (50 ml) was reduced with LAH (1·5 g) in the usual way to afford IIId (2·2 g) as an oil. The alcohol (0·93 g) was dissolved in pyridine (60 ml) and treated with OsO_4 (0·95 g) at room temp for 15 hr. A solution of $NaHSO_3$ (4 g) in pyridine (10 ml) and water (60 ml) was added, and after 4 hr the mixture was extracted with $CHCl_3$ to give a neutral gum (1·03 g). Filtration through alumina (Act IV; 40 g) in ether and crystallization from benzene–light petroleum gave needles (0·85 g) of *eperuane-8β-15,20-triol* (Va), m.p. 98–99°, $[\alpha]_D$ +8° (*c*, 5·3, EtOH). (Found: 73·5; H, 11·6. $C_{30}H_{52}O_3$ requires: C, 73·6; H, 11·7%.)

Eperu-8(20)-ene (IIIe)

Eperu-8(20)-en-15-ol (IIId; 0·77 g) in pyridine (50 ml) was cooled to 0° and toluene-*p*-sulphonyl chloride (1·5 g) added. After 15 hr at 0° the neutral product was isolated in the usual way with ether to give the toluene-*p*-sulphonate (0·98 g) as an oil. To a solution of the crude tosylate in tetrahydrofuran (50 ml) was added LAH (1·5 g) and the mixture heated under reflux for 16 hr. Decomposition of the excess reagent with water and isolation with ether gave an oil (0·57 g) which was dissolved in light petroleum and filtered through neutral alumina (Act I, 20 g) yielding *eperu-8(20)-ene* (IIIe; 0·46 g) as a colourless oil, b.p. 115–116°/0·2 mm. $[\alpha]_D$ −39° (*c*, 7·3). (Found: C, 86·9; H, 12·9. $C_{30}H_{50}$ requires: C, 86·9; H, 13·1%.)

Eperuane-8β-20-diol (Vb)

The hydrocarbon (IIIe; 0·26 g) in pyridine (15 ml) was treated with OsO_4 (0·40 g) at room temp for 16 hr. Sodium bisulphite (3 g) in water (30 ml) and pyridine (20 ml) was added and after 2 hr the neutral product (0·30 g) was isolated with ether and recrystallized from light petroleum to give the *diol* (Vb) as needles, m.p. 121–122°, $[\alpha]_D$ +7° (*c*, 2·0) . (Found: C, 77·7; H, 12·3. $C_{30}H_{52}O_2$ requires: C, 77·4; H, 12·3%.)

15,18-*Diacetoxyeperuane-8β-ol* (VIb)

The triol (VIa; 5·10 g) in pyridine (30 ml) was treated with acetic anhydride (18 ml) at room temp for 18 hr. Isolation in the usual way with ether gave an oil (6·6 g) which was chromatographed on Woelm neutral alumina (Act V; 100 g). Elution with light petroleum and benzene–light petroleum (1:9 and 1:3) gave VIb (6·0 g) as a viscous oil, $[\alpha]_D$ −22° (*c*, 8·0). (Found: C, 70·2; H, 10·2. $C_{34}H_{42}O_6$ requires: C, 70·2; H, 10·3%.) The diacetate was hydrolysed with 10% KOH in MeOH (3 hr reflux) to regenerate the triol (VIa) as needles (from benzene), m.p. and mixed m.p. 121–122°, $[\alpha]_D$ −13° (*c*, 4·3, EtOH).

Oxidation of eperuane-8β,15,18-triol (VIa)

The triol (VIa; 3·06 g) in acetone (100 ml) was cooled to 0° and the Jones reagent[6] (6·90 ml; calc. for 6 equiv. per mole = 7·03 ml) was added dropwise with swirling over 1 hr. The mixture was diluted with water and extracted with ether. The organic layer was washed with 2% KOHaq, the alkaline solution acidified and extracted with ether to give VIc (2·80 g) as an oil. The *oxime* crystallized from aqueous MeOH as needles, m.p. 98–100°, $[\alpha]_D$ −18° (*c*, 2·3, EtOH), (Found: C, 68·0; H, 9·8; N, 3·8. $C_{30}H_{55}O_4N$ requires: C, 68·0; H, 10·0; N, 4·0%.)

Wolff-Kishner reduction of 8β-*hydroxy*-18-*oxo-eperuan*-15-*oic acid* (VIc)

The oxo-acid (VIc; 1·0 g) in EtOH (10 ml) and diethylene glycol (60 ml) was treated with hydrazine hydrate (100%; 6 ml) under reflux for 1·5 hr. KOH (5 g) was added, the solution concentrated to an internal temp of 208° and heated under reflux for 4 hr. Dilution with 5% HClaq and isolation with ether gave the acid which was methylated directly with diazomethane in ether. Two recrystallizations from light petroleum at −15° gave needles (0·45 g) of Ic, m.p. and mixed m.p. 73–74°.

Dehydration of 15,18-*diacetoxyeperuane*-8β-*ol* (VIb)

To VIb (5·2 g) in pyridine (100 ml) was added $POCl_3$ (15 ml) in pyridine (50 ml). After 18 hr at 0° the mixture was poured into ice–$NaHCO_3$aq with stirring. The neutral product (4·7 g) was isolated with ether and chromatographed on alumina (neutral, Act III; 50 g). Elution with light petroleum and benzene–light petroleum (1:9 and 1:1) gave the olefin (4·10 g), the NMR spectrum of which showed it to be a mixture of double bond isomers containing ca 68% of the exocyclic isomer and 20% of the 7(8)-isomer.

The diacetate mixture (4·0 g) was saponified with 10% KOH in MeOH (3 hr reflux) and the resulting crystalline diols (3·0 g) repeatedly recrystallized from benzene–light petroleum to give *eperu*-8(20)-*ene*-15,18-*diol* (VIIa; 1·2 g), as needles, m.p. 101–102°, $[\alpha]_D$ −43°, (*c*, 5·8, EtOH) $\nu_{max}^{CCl_4}$ (0·006 M) = 3640, 3078, 1641 cm^{-1}. (Found: C, 77·9; H, 11·7. $C_{20}H_{36}O_2$ requires: C, 77·9; H, 11·8%.)

Derivatives of 15,16-*dihydroxyeperu*-8(20)-*en*-18-*oic acid* (VIIIa)

The *methyl ester* (VIIIb), prepared with diazomethane in ether at 0° in the usual way, crystallized from benzene–light petroleum as rosettes of needles, m.p. 90–91°, $[\alpha]_D$ −34° (*c*, 3·7), $\nu_{max}^{CS_2}$ = 3080, 1730, 1645, 890 cm^{-1}. (Found: C, 71·4; H, 10·1. $C_{21}H_{36}O_4$ requires: C, 71·6; H, 10·3%).

The IR spectrum (CCl_4; 0·004 M) had hydroxyl absorption at 3634 and 3470 (concentration independent) cm^{-1}. In an 0·01 M solution, intermolecular bonded OH at 3295 cm^{-1} was also present.

The ester (8 g) was saponified by heating with KOH (25 g) in n-butanol (100 ml) and water (5 ml) under reflux for 6 hr. Dilution with water and isolation of the acid fraction (7·5 g) with $CHCl_3$ gave the acid (VIIIa) as plates (from acetone), m.p. and mixed m.p. 152–153°.

The *diacetate* of VIIIa, prepared with acetic anhydride in pyridine at room temp overnight, was an oil, $\nu_{max}^{CS_2}$ = 3080, 1743, 1692, 1645, 890 cm^{-1}. The *cyclohexylamine salt* of the diacetate, prepared in hot acetone–light petroleum, was recrystallized from the same solvent system to give rosettes of needles, m.p. 141–144° (cap., dec), $[\alpha]_D$ −17° (*c*, 4·2), ν_{max}^{Nujol} = 3080, 2235, 1742, 1645, 900 cm^{-1}. (Found: C, 69·2; H, 9·7; N, 3·0. $C_{30}N_{51}O_6N$ requires: C, 69·1; H, 9·9; N, 2·7%.)

Hydrogenation of 15,16-*dihydroxyeperu*-8(20)-*en*-18-*oic acid* (VIIIa)

The olefin (VIIIa; 4·5 g) in MeOH (85 ml) was shaken with H_2 at room temp in the presence of Adams' catalyst (0·42 g). One mole H_2 was absorbed rapidly and after 2 hr the catalyst was filtered off and the solvent removed from the filtrate *in vacuo* to afford the crystalline dihydroacid, m.p. 134–141°. One recrystallization from aqueous MeOH gave needles (3·30 g), m.p. 138–142°, which on further repeated recrystallization from the same solvent system afforded 15-16-*dihydroxy*-8β(H)-*eperuan*-18-*oic acid* (VIIIc) as needles, m.p. 143–144°, $[\alpha]_D$ −43° (*c*, 2·8, EtOH), ν_{max}^{Nujol} = 3400, 3250, 1690, pK_{MCS}^{*} 7·98, equiv. wt. 339, (Found: C, 70·3; H, 10·7. $C_{20}H_{36}O_4$ (340·5) requires: C, 70·5: H, 10·7%.)

Reduction of methyl 15,16-*dihydroxyeperu*-8(20)-*en*-18-*oate* (VIIIb)

The ester (3·2 g) in ether (100 ml) was heated under reflux with LAH (3 g) for 1 hr. Isolation of the neutral product (2·95 g) with $CHCl_3$ in the usual way and recrystallization from benzene afforded *eperu*-8(20)-*ene*-15,16,18-*triol* (VIIId) as dimorphic needles, m.p. 101–102° and 122–123°, $[\alpha]_D$ −49° (*c*, 3·1, EtOH), ν_{max}^{Nujol} = 3350, 3300, 3085, 1645, 885 cm^{-1}. (Found: C, 73·8; H, 11·1. $C_{20}H_{38}O_3$ requires: C, 74·0; H, 11·2%.)

Reduction of the acid (VIIIa) with LAH in dioxan (4 hr reflux) similarly gave the triol (VIIId) as needles (from benzene), m.p. and mixed m.p. 122–123°.

The *triacetate* of VIIId, prepared with acetic anhydride in pyridine at room temp overnight, was an oil, $\nu_{max}^{CS_2}$ = 3080, 1745, 1645, 1230, 1035, 890 cm^{-1}. (Found: C, 69·0; H, 9·7. $C_{26}H_{42}O_6$ requires: C, 69·3; H, 9·4%.)

Hydrogenation of eperu-8(20)-ene-15,16,18-triol (VIIId)

The triol (VIIId; 1·52 g) in MeOH (50 ml) was hydrogenated over Adams' catalyst (0·12 g) at room temp. After 2 hr the catalyst was removed and the solvent evaporated *in vacuo*. The crude dihydrotriol (1·57 g), m.p. 134–140°, was crystallized once from benzene–MeOH to afford plates (0·98 g), m.p. 141–143°. A second crop (0·30 g) had m.p. 124–134°. Repeated recrystallization of the first crop from aqueous MeOH gave 8β(H)-*eperuane*-15,16,18-*triol* (VIIIe) as needles, m.p. 143–144°, $[\alpha]_D$ −52° (*c*, 2·9, EtOH), ν_{max}^{Nujol} = 3250, 3190, 1040 cm^{-1}. (Found: C, 73·7; H, 11·6. $C_{30}H_{58}O_3$ requires: C, 73·6; H, 11·7%.)

15,16-*Ethylidenedioxy*-8β(H)-*eperuan*-18-*oic acid* (IXa)

The diol (VIIIc; 0·36 g) was finely powdered, suspended in dry ether (25 ml) containing paraldehyde (2 ml), and conc HClaq added (4 drops). The mixture was shaken until dissolution occurred (15 min) and left at room temp 2 hr with occasional shaking. The solution was washed with water, dried, and the solvent evaporated *in vacuo* to afford the ethylidene derivative (IXa) as a viscous oil. The *cyclohexylamine salt* crystallized from acetone–light petroleum as fine needles, m.p. 190–193° (cap, dec), $[\alpha]_D$ −32° (*c*, 2·1). (Found: C, 72·1; H, 11·0; N, 3·1. $C_{38}H_{61}O_4N$ requires: C, 72·2; H, 11·0; N, 3·0%.)

15,16-*Ethylidenedioxy*-8β(H)-*eperuan*-18-*al* (IXc)

The triol (VIIIe; 0·78 g) was shaken in ether (40 ml) with paraldehyde (1·5 ml) and conc. HClaq (4 drops) for 2 hr. The solution was washed with $NaHCO_3$aq, water, dried and the solvent evaporated *in vacuo*. The crude product was filtered through Woelm neutral alumina (Act III; 10 g) in benzene to give the ethylidene derivative (IXb; 0·50 g) as an oil. The 3,5-*dinitrobenzoate*, prepared in pyridine at room temp overnight, crystallized from aqueous MeOH as needles, m.p. 112–114° (Found: N, 5·1. $C_{39}H_{44}O_8N_2$ requires: N, 5·1%).

The alcohol (IXb; 0·3 g) in pyridine (10 ml) was treated with CrO_3 (0·50 g) in pyridine (10 ml). After 2 hr at room temp the mixture was reduced with MeOH (5 ml), diluted with 2% KOHaq and the *aldehyde* (IXc; 0·23 g) isolated with ether as an oil. The *semicarbazone* was prepared by treating the aldehyde (0·2 g) in pyridine (2 ml) and MeOH (5 ml) with a mixture of semicarbazide hydrochloride (0·6 g) and dry NaOAc (0·7 g) in MeOH (10 ml). After 2 days at room temp, the solution was concentrated, diluted with water and the precipitate recrystallized from aqueous MeOH at 0° to afford the derivative as fine crystals, m.p. 88–90° (cap), $[\alpha]_D$ −37° (*c*, 1·4). (Found: C, 68·0; H, 10·1; N, 9·9. $C_{33}H_{61}O_3N_3$ requires: C, 67·8; H, 10·1; N, 10·3%).

Dehydration of 15,16-*dihydroxy*-8β(H)-*eperuan*-18-*oic acid* (VIIIc)

To the diol (VIIIc; 0·695 g) in benzene (100 ml) was added *p*-toluenesulphonic acid monohydrate (0·70 g), the solution concentrated to 50 ml and then heated under reflux for 4 hr. The solution was washed with water, dried and the solvent removed to give a resin (0·66 g) which was chromatographed on silicic acid (Act I; 14 g). Elution with ether–benzene (1:9) gave 15,16-*oxido*-8β(H)-*eperuan*-18-*oic acid* (IXd; 0·627 g) which crystallized from aqueous MeOH as needles, m.p. 123–124°, $[\alpha]_D$ −50° (*c*, 3·1), $\nu_{max}^{CS_2}$ = 1695, 1055 cm^{-1}. (Found: C, 74·3; H, 10·5. $C_{30}H_{54}O_3$ requires: C, 74·5; H, 10·6%.) The IR (CS_2) and NMR spectra were identical with those of hexahydropolyalthic acid.[2,3]

The *cyclohexylamine salt* of IXd crystallized from acetone–light petroleum as needles, m.p. 191–195° (cap; dec), $[\alpha]_D$ −29° (*c*, 4·6), ν_{max}^{Nujol} = 2210, 1630, 1053 cm^{-1}. (Found: C, 74·1; H, 11·0; N, 3·4. $C_{36}H_{67}O_3N$ requires: C, 74·1; H, 11·2; N, 3·3%.) The salt had m.p. 189–193° (cap, dec), on admixture with the *cyclohexylamine salt of hexahydropolyalthic acid* of m.p. 186–190° (cap, dec), $[\alpha]_D$ −26° (*c*, 7·0). (Found: C, 74·5; H, 11·1; N, 3·6%.) The two salts had almost identical IR spectra (Nujol).

Isomerization and dehydration of methyl 15,16-*dihydroxyeperu*-8(20)-*en*-18-*oate* (VIIIb)

The olefin (VIIIb; 0·92 g) in MeOH (50 ml) was heated under reflux with conc H_2SO_4 (4·0 ml) for 8 hr. Dilution with water and isolation of the neutral product (0·85 g) with ether gave an oil which was chromatographed on neutral alumina (Act III; 25 g). Elution with light petroleum and benzene–light petroleum (1:19, 1:9 and 1:1) gave a colourless oil (0·585 g). The IR spectrum (CS_2) contained no hydroxyl absorption near 3600 cm^{-1}, no vinylidene absorption and a weak band at

1685 cm^{-1} attributed to the tetrasubstituted double bond. The methyl ester (0·54 g) was hydrolysed by heating with KOH (10 g) in butanol (40 ml) and water (4 ml) under reflux for 7 hr. Isolation of the acidic product (0·40 g) with ether and crystallization from aqueous MeOH gave 15,16-*oxidoeperu*-8-*en*-18-*oic acid* (IVb) as needles, m.p. 127–128°, $[\alpha]_D$ −87° (*c*, 4·7). (Found: C, 75·2; H, 9·9. $C_{30}H_{52}O_3$ requires: C, 75·0; H, 10·1%.)

Hydrogenation of 15,16-*oxidoeperu*-8-*en*-18-*oic acid* (IVb)

The olefin (80 mg) in acetic acid (10 ml) was shaken with H_2 at room temp in the presence of Adams' catalyst (20 mg) for 4 hr. The catalyst was filtered off, the filtrate diluted with water and the acidic product (78 mg) isolated with ether. Repeated crystallization from aqueous MeOH gave needles of IXd, m.p. and mixed m.p. 120–122°.

Oxidation of 15,16-*dihydroxy*-8β(H)-*eperuan*-18-*oic acid* (VIIIc)

The diol (0·28 g) in acetone (25 ml) was treated with the Jones reagent[6] dropwise at room temp and the mixture left with a slight excess for 2 hr. MeOH (3 ml) was added, the solution diluted with water and the oily γ-lactone mixture isolated with $CHCl_3$, $\nu_{max}^{CS_2}$ = 1785 (γ-lactone), 1741 (carboxyl monomer), 1695 (carboxyl dimer) cm^{-1}. The *cyclohexylamine salt* crystallized from acetone–light pertoleum as needles, m.p. 182–184° (cap; dec), $[\alpha]_D$ −27° (*c*, 1·3). ν_{max}^{Nujol} = 1770, 1636 cm^{-1}. (Found: C, 71·7; H, 10·4; N, 3·1. $C_{36}H_{65}O_4N$ requires: C, 71·7; H, 10·4; N, 3·2%.)

Oxidation of methyl 15,16-*dihydroxyeperu*-8(20)-*en*-18-*oate* (VIIIb)

The diol (5·660 g) in acetone (200 ml) was treated with the Jones reagent[6] dropwise at room temp over a period of 2 hr with swirling. The solution took up 8·04 ml of reagent (calc. for 4 equiv./mole = 8·05 ml). Several drops of excess reagent were added and after 15 min the mixture was treated with MeOH (5 ml), diluted with water and extracted with ether. The organic layer was washed with water, $NaHCO_3$aq, water, dried and the solvent removed to give a neutral oil (5·4 g) which partly crystallized. Chromatography on Woelm neutral alumina (Act III; 50 g) and elution with benzene–light petroleum (1:19, 1:9 and 1:3) gave the oily lactone mixture (4·50 g) which partly crystallized. Repeated crystallization from benzene–light petroleum and then aqueous MeOH gave the *γ-lactone* (Xb; 1·60 g) as needles, m.p. 119–120°, $[\alpha]_D$ −22° (*c*, 4·1), $\nu_{max}^{CS_2}$ = 3080, 1783, 1732, 1645, 890 cm^{-1}. (Found: C, 72·7; H, 9·5. $C_{31}H_{52}O_4$ requires: C, 72·4; H, 9·3%.) The IR spectrum of the crude oxidation product was very similar to that of the purified γ-lactone (Xb).

Reduction of the γ-lactone mixture with LAH in ether (5 hr reflux) gave the triol (VIIId) as needles (from benzene), m.p. and mixed m.p. 121–122°.

Eperu-8(20)-*ene* (IIIe) *from eperu*-8(20)-*ene*-15,16,18-*triol* (VIIId)

To a solution of toluene-*p*-sulphonyl chloride (4·65 g) in pyridine (20 ml), cooled to −15°, was added dropwise with stirring, a solution of VIIId (1·50 g) in pyridine (20 ml) over a period of 4 hr. After a further 18 hr at −20°, water was added slowly with cooling, the mixture diluted with water and the neutral product (3·20 g) isolated with ether to give the crude tritosylate as an oil. Integration of the NMR spectrum of the product showed it to contain at least 95% of the tritosylate.

A comparative experiment, in which the acid chloride was added all at once to a solution of the triol in pyridine at 0° and the mixture left 16 hr at 0° and worked up as above, gave a mixture. The NMR spectrum showed it to contain only ca. 30% of the tritosylate and 70% of the 15,16-oxido-18-tosylate derivative.

The crude tritosylate (3·15 g) in ether (75 ml) was added slowly over a period of 1·5 hr to a mixture of LAH (6 g) and ether (250 ml) heated under reflux. After a further 15 hr reflux, the excess reagent was destroyed with ice and the neutral product (1·35 g) chromatographed on alumina (Act I; 20 g). Elution with light petroleum gave IIIe (0·140 g), which was converted to Vb with OsO_4 in pyridine[18] as described previously. The diol crystallized as needles (from light petroleum), m.p. and mixed m.p. 121–122°. Elution of the alumina column with benzene gave an oil (0·46 g), the NMR spectrum of which showed it to be VIIb. Treatment of this with OsO_4 (0·4 g) in pyridine (15 ml) at room temp for 19 hr, and working up with $NaHSO_3$[18] as previously described gave 18-*toluene*-p-*sulphonyloxy*-*eperuane*-8β-20-*diol* (Vc) as needles (from benzene–light petroleum), m.p. 103–104°, $[\alpha]_D$ −19° (*c*, 2·4). (Found: C, 67·2; H, 9·3; S, 6·7. $C_{37}H_{44}O_5S$ requires: C, 67·5; H, 9·2; S, 6·7%.)

Degradation of the γ-lactone (Xb) *to the diester* (XIb)

To a solution of sodium benzylmercaptide in dimethylformamide (10 ml),[8] prepared from Na (0·830 g) and benzyl mercaptan (5·0 ml), was added a solution of Xb (2·58 g) in DMF (20 ml) and the mixture heated at 95° on a steam bath for 15 hr under dry N_2. The solution was then diluted with water, extracted thoroughly with ether (discarded), the aqueous layer acidified and the acidic product (XIa) isolated with ether. The solvent was removed *in vacuo* and the residual diacid (3·20 g) methylated directly with diazomethane in ether to give the diester, $\nu_{max}^{CS_2} = 1732\ cm^{-1}$.

The crude benzylthioether (3·25 g) was desulphurized directly. The Raney Ni (W-4; 60 ml of slurry) was first deactivated by heating under reflux with ethyl acetate (200 ml) for 2 hr, then acetone (200 ml) for 3 hr and the acetone replaced by EtOH (150 ml). The thioether in EtOH (50 ml) was then added and the mixture heated under reflux for 2·5 hr. The catalyst was filtered off, washed with MeOH and the filtrate concentrated, diluted with water and the neutral product (2·45 g) isolated with ether in the usual way. Chromatography on Woelm neutral alumina (Act I; 45 g) and elution with benzene–light petroleum (1:9, 1:3 and 1:1) and benzene gave the *dimethyl ester* (XIb; 1·60 g) as a mobile oil. The diester (1·14 g) was rechromatographed on silica gel-$AgNO_3$[15] (200 g). Elution with ether–light petroleum (1:6, 1:4 and 1:3) gave oils (1·13 g); the NMR and IR spectra of all fractions were identical to those of the starting diester, $[\alpha]_D$ −26° (*c*, 4·0). The mass spectrum was virtually indistinguishable from that quoted for dimethyl pinifolate[21] (XIII), $\nu_{max}^{CCl_4} = 3080, 2940, 2865, 1734, 1730, 1642, 1460, 1443, 1432, 1385, 1370, 1242, 1197, 1170, 1157, 1145, 1128, 1101, 1060, 1044, 891\ cm^{-1}$. (Found: mol. wt., 364 (mass spectrometer). $C_{22}H_{36}O_4$ requires: mol. wt., 364·5.)

Eperu-8(20)-*ene*-16,18-*diol* (XIc)

The dimethyl ester (XIb; 0·80 g) in ether (50 ml) was heated under reflux with LAH (1·5 g) for 1 hr. Isolation of the neutral product with ether and crystallization from benzene–light petroleum gave XIc as plates or needles, m.p. 86–87°, $[\alpha]_D$ −45° (*c*, 3·2, EtOH), $\nu_{max}^{Nujol} = 3330, 3085, 1645, 1050, 890\ cm^{-1}$. (Found: C, 77·9, H, 11·6. $C_{20}H_{36}O_2$ requires: C, 77·9; H, 11·8%.)

8-*Oxo*-20-*nor*-*eperuan*-16-*oic acid* (XII)

The diol (XIc; 0·680 g) in acetone (150 ml) was cooled to 0° and the Jones reagent[6] (1·70 ml; calc. for 6 equiv/mole = 1·65 ml) added dropwise with swirling over a period of 1 hr. Dilution with water and isolation with ether gave XId (0·60 g). $\nu_{max}^{CS_2} = 3080, 2687, 1730, 1705, 1645, 890\ cm^{-1}$.

The crude oxo-acid in EtOH (5 ml) and diethylene glycol (75 ml) was treated with hydrazine hydrate (5 ml; 100%) under reflux for 2 hr. KOH (8 g) was added, the mixture concentrated to an internal temp of 208° and heated under reflux for 4 hr. Dilution with 2% HClaq and isolation with ether gave eperu-8(20)-en-16-oic acid (0·49 g) as an oil, $\nu_{max}^{CS_2} = 3080, 1705, 1645, 890\ cm^{-1}$. The unsaturated acid in methyl acetate (50 ml) was cooled to −70° and the solution saturated with O_2. Removal of excess O_2 and evaporation of the solvent *in vacuo* gave an oil which was heated with water (25 ml) on a steam bath for 1 hr and then the product (0·45 g) isolated with ether. Chromatography on silicic acid (Act I; 25 g) and elution with ether–benzene (1:9 and 1:2) gave the *keto-acid* (XII; 0·34 g) as an oil, $\nu_{max}^{CS_2} = 1710\ cm^{-1}$. The *oxime* crystallized from aqueous MeOH as needles, m.p. 180–182° with a change in crystalline form to prisms at 170°, $[\alpha]_D$ −90° (*c*, 1·8, dioxan). (Found: C, 70·8; H, 10·2. $C_{19}H_{33}O_3N$ requires: C, 70·6; H, 10·3%.)

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