CHEMISTRY LETTERS, pp. 881-884, 1982. © The Chemical Society of Japan 1982

FERN CONSTITUENTS: NEW CYCLOARTANE TRITERPENOIDS, (24*R*)-CYCLOLAUDENYL ACETATE AND (24*R*)-CYCLOMARGENYL ACETATE, AND THEIR CORRESPONDING ALCOHOLS AND KETONES, ISOLATED FROM POLYPODIUM FORMOSANUM

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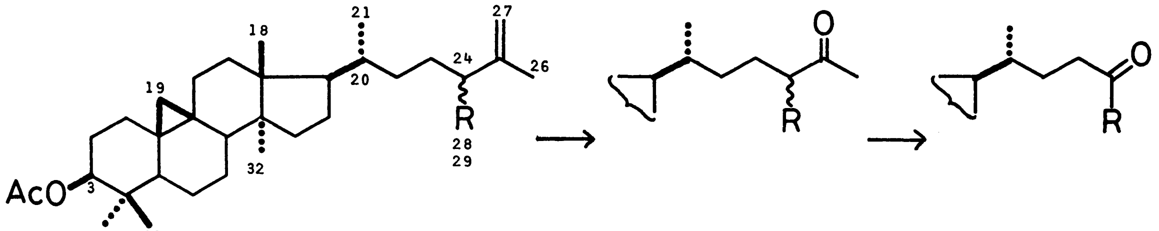
New tetracyclic triterpenoids, (24*R*)-cyclolaudenyl acetate(Ia) and (24*R*)-cyclomargenyl acetate(Ib), and their corresponding alcohols(IIa, IIb) and ketones(IIIa,IIIb) were isolated from the rhizomes of *Polypodium formosanum*. The structure of Ia and Ib were deduced by chemical and spectroscopic methods to be (24*R*)-24-methylcycloart-25-en-3β-yl acetate and (24*R*)-24-ethylcycloart-25-en-3β-yl acetate, respectively.

From the rhizomes of a Formosan fern, *Polypodium formosanum* BAKER(=*Marginaria formosana* NAKAI, "Taiwan-aone-kazura", Polypodiaceae), two new triterpenoids, named (24*R*)-cyclolaudenyl acetate(Ia) and (24*R*)-cyclomargenyl acetate(Ib)[1)] (0.68% of the dried materials, ca.1:1 mixture) were obtained together with their corresponding 3β-alcohols, IIa and IIb(0.002%, 1:1), and ketones, IIIa and IIIb(0.002%, 1:1). This paper concerns full structural elucidations of these compounds.

Chloroform-methanol extraction followed by chromatography on silica gel gave respective mixture of Ia and Ib, IIa and IIb, and IIIa and IIIb. Separation of these homologues was rather difficult and achieved by repeated chromatography on silica gel impregnated 20% silver nitrate, preparative GLC, and/or HPLC on reversed phase column.

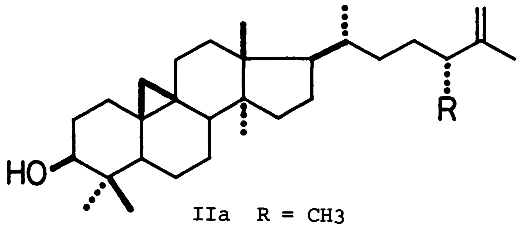
For the compound Ia, mp.127-128°, $[\alpha]_D^{24}$ +53.5°($CHCl_3$), elemental analysis(C,82.07; H,11.47%) and a molecular ion peak at m/z 482.4149 in the MS spectrum indicated



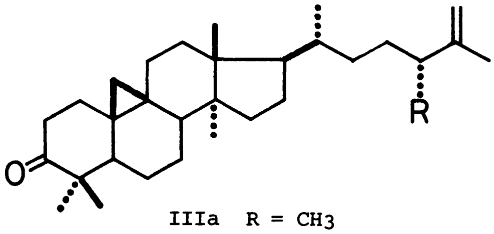
Ia R = ••••CH_3 (20*R*,24*R*)
Ib R = ••••C_2H_5(20*R*,24*R*)
Ic R = ▬CH_3 (20*R*,24*S*)

IVa R = ••••CH_3
IVb R = ••••C_2H_5
IVc R = ▬CH_3

Va,Vc R = CH_3
Vb R = C_2H_5



IIa R = CH_3
IIb R = C_2H_5



IIIa R = CH_3
IIIb R = C_2H_5

the molecular formula $C_{33}H_{54}O_2$. The IR spectrum of Ia showed presence of a terminal methylene ($\nu^{KBr}_{cm^{-1}}$ 3070, 1648, 885), an acetoxyl (1735, 1250, 1026), and a cyclopropane methylene (3040). The MS (TABLE I)[2] and the H-NMR (TABLE II, 100MHz) spectra of Ia were almost superimposable to those of known cyclolaudenyl acetate (Ic), mp. 121°, $[\alpha]^{24}_{D}$ +55°, of opium origin,[3] but a mixture of Ia and Ic melted at below 115° to provide both compounds being not identical.

Careful ozonolysis of Ia in a hexane solution at -60°C followed by treatment with zinc powder and acetic acid at room temperature, or osmium tetraoxide and lead tetraacetate oxidation in neutral condition afforded a methyl ketone (IVa),[4] IR: 1730, 1242, 1022; 1712; 3040. The MS (TABLE I, appropriate shifts to mass numbers higher by two for molecular ion and fragment ions containing the side chain) and the ^{1}H-NMR (TABLE II, down field shifts of C26 protons to δ2.127s and C28 protons to δ1.084d) spectra of IVa clearly indicated the presence of methyl ketone structure at the end of side chain. IVa was further oxidized with oxygen in *t*-butanol-tetrahydrofuran with potassium *t*-butoxide followed by acetylation to give another methyl ketone (Va), mp. 167°, $[\alpha]^{24}_{D}$ +51.6°, IR: 1727, 1250, 1712; 3040. By the MS (further shifts to mass numbers lower by 28 for molecular ion and ions containing the side chain) and the ^{1}H-NMR (down field shift of C28 protons to δ2.139s) spectra, the structure of Va was confirmed.

On the other hand, Ic was also oxidized in the same way as Ia, to afford the first methyl ketone (IVc)[4] and the second one (Vc), mp. 168°. $[\alpha]^{24}_{D}$ +52.4°. As the identity of Va and Vc was proved by mixture melting point determination, GLC, IR, ^{1}H-NMR and MS, Ia was established to have the same structure as Ic except the absolute configuration at C24.

The compound Ib, mp.144-145°, $[\alpha]^{24}_{D}$ +50.5°, was indicated to have the molecular formula $C_{34}H_{56}O_2$ (M^+ 496.4292; C,82.07; H,11.47%). The IR spectrum (3070, 1645, 885; 1730, 1250, 1026; 3040) was quite resemble to that of Ia. The fact that chemical shifts of the protons in Ib attached to C3, C18, C19, C21, C30, C31 and C32 were almost identical with those of Ia and Ic (TABLE II), suggested Ib has the same structure of cycloartane nucleus including the configuration at C20. Proton signals of Ib were remarkably different from that of Ia or Ic at methyl(C26), terminal methylene (C27, also shown in Fig.1) and at a triplet methyl[C29, instead of a doublet methyl(C28) in Ia or Ic]. Similarly, Ib provided the MS spectrum with shifts to mass numbers higher by 14 for molecular ion and ions containing the side chain, and presence of an extra carbon in side chain were suggested.

Oxidation of Ib in the same way as Ia, gave a methyl ketone (IVb),[4] IR: 1732, 1250, 1026; 1712; 3050. The MS (TABLE I, shifts to mass numbers higher by two for molecular ion and fragment ions containing the side chain) and the ^{1}H-NMR (TABLE II, down field shifts of C26 protons to δ2.108s and C29 protons to δ0.867t) spectra of IVb clearly demonstrated its structure. IVb was further oxidized to give an ethyl ketone (Vb), IR: 1730, 1250, 1025; 1710; 3050. The structure of Vb was also confirmed by the MS (TABLE I, further shifts to mass numbers lower by 28 for molecular ion and ions with side chain) and the ^{1}H-NMR (TABLE II, a clear triplet at δ1.045 assignable for C29 protons) spectra.

Proof for the configuration at C24 of Ia and Ib was accomplished by measuring CD curves of IVa and IVb, comparing with that of IVc, and also with those of IVa' and

TABLE I. MS Spectra (Hitachi RMS-4)

	M^+	M^+-15	a	a-15	a-43	a-69	b	c	d	e
Ia	482(31)	467(13)	422(100)	407(39)	379(24)	353(16)	300(50)	357(10)	297(27)	175(50)
Ib	496(31)	481(12)	436(100)	421(37)	393(18)	367(15)	314(42)	357(10)	297(23)	175(42)
Ic	482(30)	467(13)	422(100)	407(56)	379(24)	353(19)	300(46)	357(19)	297(23)	175(42)
IIa	440(57)	425(62)	422(100)	407(80)	379(25)	353(22)	300(64)	315(20)	297(26)	175(59)
IIb	454(53)	439(48)	436(90)	421(100)	393(27)	367(18)	314(40)	315(21)	297(18)	175(39)
IIIa	438(100)	423(19)			395(7,f)		300(28)	313(43)		175(19)
IIIb	452(100)	437(19)			409(4,f)		314(33)	313(42)		175(19)
IVa	484(5)	469(2)	424(55)	409(44)	381(18)	355(29)	302(44)	357(18)	297(42)	175(100)
IVb	498(12)	483(9)	438(100)	423(62)	395(21)	369(23)	316(56)	357(20)	297(45)	175(100)
Va	456(8)	441(8)	396(71)	381(62)	353(19)	327(19)	274(46)	357(9)	297(30)	175(100)
Vb	470(8)	455(6)	410(71)	395(52)	367(16)	341(21)	288(45)	357(12)	297(37)	175(100)

a) M^+-60(Ia-Ic, IVa-Vb), M^+-18 (IIa, IIb). b) a-122 (Ia-IIb, IVa-Vb), M^+-138 (Va, Vb). c) M^+-side chain. d) a-side chain. e) b-side chain. f) M^+-43.

TABLE II. Chemical Shifts (δ) for $CDCl_3$ Solution (JEOL JNM-FX 100)

	Proton(s) attached to C( )a)									
	30	31	19b)	32	18	21c)	28d) or 29e)	26	27	3α	acetyl methyl
Ia	0.845	0.887	0.334d 0.574d	0.887	0.960	0.859d	0.991d	1.639	4.659 4.669	4.54m	2.044
Ib	0.845	0.887	0.333d 0.574d	0.887	0.953	0.860d	0.806t	1.573	4.652bs 4.720m	4.54m	2.051
Ic	0.845	0.887	0.333d 0.574d	0.887	0.960	0.859d	0.991d	1.639	4.657 4.669	4.54m	2.044
IIa	0.960	0.806	0.323d 0.555d	0.882	0.960	0.847d	0.994d	1.637	4.659 4.669	3.27m	
IIb	0.963	0.806	0.321d 0.554d	0.882	0.963	0.847d	0.806t	1.566	4.655bs 4.723m	3.27m	
IIIa	1.098	1.046	0.571d 0.785d	0.897	0.990	0.870d	1.001d	1.642	4.662 4.674		
IIIb	1.098	1.044	0.576d 0.786d	0.894	0.987	0.865d	0.806t	1.573	4.666bs 4.728m		
IVa	0.848	0.887	0.337d 0.576d	0.887	0.953	0.873d	1.084d	2.127		4.55m	2.054
IVb	0.848	0.887	0.337d 0.576d	0.887	0.951	0.863d	0.867t	2.108		4.55m	2.051
Va	0.845	0.887	0.334d 0.577d	0.887	0.953	0.866d	2.139			4.55m	2.049
Vb	0.845	0.887	0.335d 0.574d	0.887	0.951	0.865d	1.045t			4.55m	2.051

a) Assignments were confirmed by $CDCl_3$-C_6D_6 solvent shifts and Lanthanide shifts. Signals otherwise stated were singlet. Coupling constants observed were b) 4.2-4.6Hz., c) 6.6-7.6Hz., d) 7.1-7.7Hz. and e) 7.6-7.9Hz.

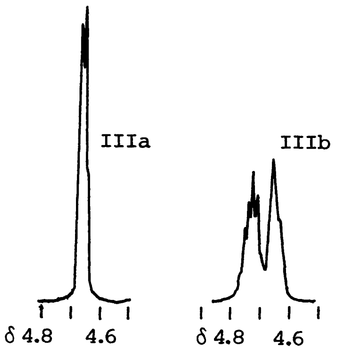


Fig.1 Terminal Methylene Signals(100MHz)
IIIa: (24*R*)-Cyclolaudenone
IIIb: (24*R*)-Cyclomargenone

IVc', which were obtained by alkaline treatment of IVa and IVc, respectively. As shown as in Fig. 2, the curves of IVa and IVb were quite resemble and found to be opposite to that of IVc centering at that of IVa' or IVc'. CD curve of (3*R*)-(-)-3,7-dimethyloctan-2-one(VI), derived from (+)-citronellol(VII) was also used as a reference to confirm the absolute configuration. Hence IVa and IVb were clearly demonstrated to have (24*R*)- and IVc (24*S*)-configuration, respectively, Ia and Ib were concluded to have (24*R*)- and Ic (24*S*)-configuration.

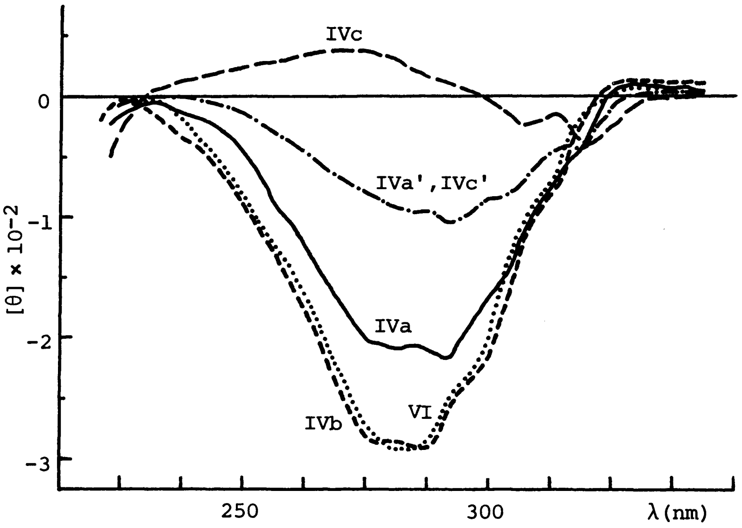
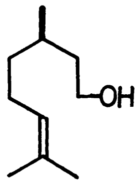
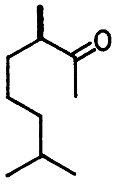


Fig. 2 CD curves of Methyl Ketones



VII



VI

The free alcohols, (24*R*)-cyclolaudenol(IIa), mp. 123-124°, $[\alpha]_D^{24}$ +36.5°($CHCl_3$), IR: 3060,1645,883; 3400,1024, 3030, and (24*R*)-cyclomargenol(IIb), mp. 134-136°, $[\alpha]_D^{24}$ +34.3°($CHCl_3$), IR: 3060,1645,886; 3400,1024; 3030, were separated as acetates, each component of which was proved to be identical with Ia and Ib. The MS(TABLE I) and the ^1H-NMR(TABLE II) spectra of IIa and IIb were also found to be quite reasonable.

The ketones, (24*R*)-cyclolaudenone(IIIa), mp. 105°, $[\alpha]_D^{24}$ +14.2°($CHCl_3$), IR: 3060, 1645,885; 1715, and (24*R*)-cyclomargenone(IIIb), mp.122-124°, $[\alpha]_D^{24}$ +13.4°($CHCl_3$), IR: 3060,1645,884; 1715, were also separated by preparative GLC from the mixture. The identity of IIIa and IIIb with the compounds obtained from IIa and IIb by CrO_3-pyridine oxidation, respectively, were proved by mixture melting point determination, IR, MS, and ^1H-NMR spectra.

The compounds Ia and Ib were also found in other Polypodiaceous ferns, such as *Polypodium niponicum* METT., *Drynaria fortunei* J.SM. and *Pseudodrynaria coronans* CHING.[1] It will be very interesting to mention that Ia and Ib have the same configuration at C24 as campesterol and sitosterol (also found in the ferns mentioned above), because a new biogenetic course, cycloartenol⟶IIa and IIb⟶campesterol and sitosterol, respectively, could be speculated in these plants.

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3) G.Ourisson, P.Crabbe, O.R.Rodig, "*Tetracyclic Triterpenes*", Hermann, Paris, 1964. The compound was kindly supplied by Prof. Ourisson, to whom our thanks are due.
4) IVa, IVb and IVc were obtained first as oil and CD curves were measured. The crystallines obtained from the oils by recrystallization have melting point at about 140° in every case, of which maximum in the CD curves was always lower than those of the oils, suggesting partial racemization occur at C24.
5) Two methylsterols, acetates of (24*R*)-4α,24-dimethylcholest-7-en-3β-ol and (24*R*)-4α-methyl-24-ethylcholest-7-en-3β-ol were also isolated from the rhizome of *Polypodium niponicum*. The presence of these methylsterols may also support the hypothesis.

(Received March 20, 1982)