

4-Epicycloeucalenone and 4-Epicyclomusalenone: Two 3-Oxo-28-norcycloartanes from the Fruit Peel of *Musa sapientum* L.

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Two 3-oxo-28-norcycloartane-type triterpenes, 4-epicycloeucalenone and 4-epicyclomusalenone, and two known 3-oxo-29-norcycloartanes, cycloeucalenone and cyclomusalenone, were isolated from the *n*-hexane extract of the fruit peel of *Musa sapientum* L. (banana). The structures of the 28-norcycloartanes were determined by spectroscopic and chemical methods.

Key words *Musa sapientum*; banana peel; 28-norcycloartane-type triterpene; Musaceae

The 3-oxotriterpene fraction is a major lipid component of peel of banana (*Musa sapientum* L.).¹ This fraction contains two 3-oxo-29-norcycloartane-type triterpenes, viz., cycloeucalenone [24-methyl-29-norcycloart-24(24¹)-en-3-one; **2a**] and cyclomusalenone [(24*S*)-24-methyl-29-norcycloart-25-en-3-one; **2b**], as predominant components.^{1,2} We reinvestigated the 3-oxotriterpene fraction and report the isolation and structure elucidation of the 4β-methyl isomers, **1a** and **1b**, of the major oxo-steroids, **2a** and **2b**.

Column chromatography over silica gel of the *n*-hexane extract (extraction was done at room temp.) of lyophilized banana peel followed by reverse phase HPLC yielded **1a**, **1b**, **2a**,²⁾ and **2b**.²⁾ Gas liquid chromatography (GLC) analysis showed these compounds to be pure.

The high-resolution mass spectrum (HR-MS) of **1a** included a molecular ion at *m/z* 424.3700 (C₃₀H₄₈O), and prominent fragment ions at *m/z* 409 (M⁺–Me), 381 (M⁺–C₃H₇), 300, 299 [loss of side chain (s.c.)], 257 [299–42 (ring D)], and 243 (299–42–CH₂). This fragmentation pattern was essentially identical to that of **2a**,¹⁾ suggesting that **1a** had the same structure as **2a** but a different stereochemistry. The structure and stereo-

chemistry of **1a** were determined by analysis of its two dimensional (2D) NMR [¹H–¹H, ¹³C–¹H correlated spectroscopies (COSYs) and heteronuclear multiple-bond correlation (HMBC)] spectra, and by comparison of its difference nuclear Overhauser effect (NOE) spectra with those of **2a**.

2a showed significant NOE correlation between [H-4β–H-19*endo*–H-8β–H-18–H-20] on the β-face, and [H-5α–H-28–H-6α] and [H-7α–H-30–H-17α] on the α-face of the molecule. In contrast, **1a** exhibited notable NOE correlation between [H-29–H-19*endo*–H-8β–H-18–H-20] on the β-face, and [H-4α–H-5α–H-6α] and [H-30–H-17α] on the α-face of the molecule. This suggested that **1a** had the same stereochemistry as **2a** except for the configuration at C-4. Thus **1a** appeared to be the C-4 epimer of **2a**, viz. 4-epicycloeucalenone [24-methyl-28-norcycloart-24(24¹)-en-3-one]. The most stable conformations of **1a** and **2a** with minimum steric energy were simulated using CAChe, and drawings³⁾ are shown in Fig. 1. The conformers are fairly consistent with the NOE correlations.

The structure of **1a** was confirmed by its chemical correlation with **2a**. On acid-catalyzed conversion,⁴⁾ **1a** gave sterically more stable **2a** accompanied by two minor

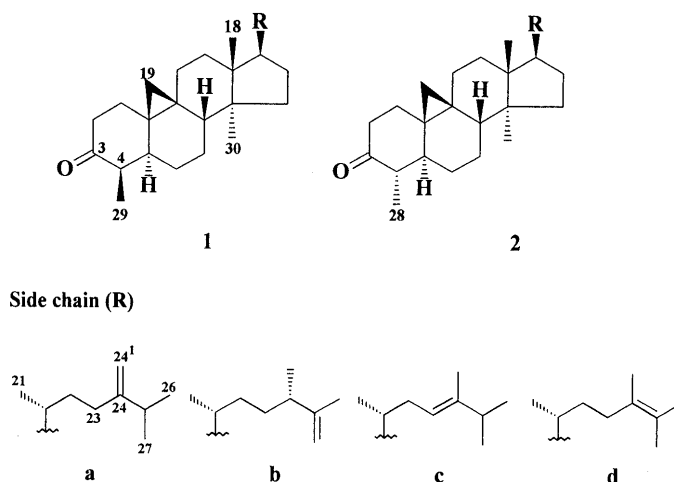


Chart 1

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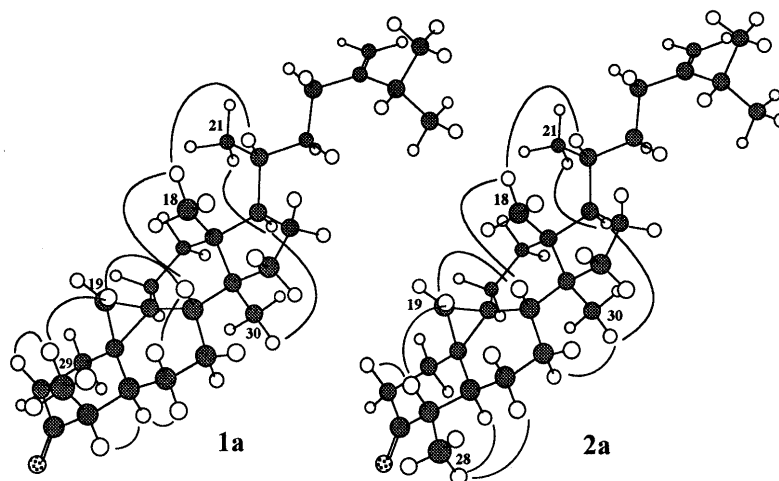


Fig. 1. CAChe Drawings and Some Representative NOE Correlations (—) for 4-Epicycloeucalenone (**1a**) and Cycloeucalenone (**2a**)
NOE correlations between *gem*-protons were omitted from the figure.

Table 1. ^{13}C - (100.62 MHz) and ^1H -NMR (400 MHz) Spectral Data (CDCl_3 , δ /ppm) for 4-Epicycloeucalenone (**1a**) and 4-Epicyclomusalenone (**1b**)^{a)}

C No.	1a		1b	
	^{13}C	^1H	^{13}C	^1H
1	33.1	1.86 (α), 1.54 (β)	33.1	1.86 (α), 1.55 (β)
2	37.4	2.28 (α), 2.65 (β)	37.4	2.26 (α), 2.65 (β)
3	216.4	—	216.4	—
4	51.7	2.44	51.7	2.43
5	42.6	2.04	42.6	2.04
6	25.7	1.37 (α), 1.18 (β)	25.8	1.36 (α), 1.18 (β)
7	25.2	1.13 (α), 1.30 (β)	25.1	1.11 (α), 1.30 (β)
8	48.2	1.62	48.2	1.62
9	20.1	—	20.1	—
10	24.8	—	24.8	—
11	26.7	2.06 (α), 1.16 (β)	26.7	2.06 (α), 1.16 (β)
12	32.8	1.66 (2H)	32.7	1.66 (2H)
13	45.4	—	45.3	—
14	48.8	—	48.8	—
15	35.6	1.33 (2H)	35.6	1.30 (2H)
16	28.1	1.95 (α), 1.32 (β)	28.0	1.90 (α), 1.31 (β)
17	52.3	1.63	52.2	1.57
18	18.1	1.00 (s)	18.0	0.99 (s)
19	29.4	0.57 (1H, d, 4.4) ^{b)} 0.78 (1H, d, 4.0) ^{c)}	29.4	0.56 (1H, d, 4.4) ^{b)} 0.77 (1H, d, 4.0) ^{c)}
20	36.1	1.41	36.0	1.35
21	18.3	0.91 (d, 6.2)	18.3	0.87 (d, 6.2)
22	35.0	1.17, 1.57	33.9	0.94, 1.33
23	31.3	1.91, 2.11	31.5	1.19, 1.43
24	156.9	—	41.6	2.10
25	23.8	2.24 (sept, 6.9)	150.2	—
26	21.9 ^{d)}	1.03 (d, 6.6)	18.6	1.64 (t, 1.1)
27	22.0 ^{d)}	1.03 (d, 6.6)	109.4	4.67 (2H, br t, 1.5)
24 ¹	106.0	4.67 (1H, br d, 1.1) 4.72 (1H, brs)	20.2	1.00 (d, 7.0)
29	12.9	1.14 (d, 7.3)	12.9	1.14 (d, 7.3)
30	19.3	0.92 (s)	19.3	0.91 (s)

a) Figures in parentheses on ^1H -NMR denote *J* values (Hz). b) *exo* methine signal. c) *endo* methine signal. d) Assignment interchangeable.

by-products formed by double bond isomerization in the side-chain, *viz.* (23*E*)-24-methyl-29-norcycloart-23-en-3-one (**2c**) and 24-methyl-29-norcycloart-24-en-3-one (**2d**).

The HR-MS of **1b** showed the molecular ion at *m/z* 424.3681 ($\text{C}_{30}\text{H}_{48}\text{O}$) accompanied by prominent fragment ions at *m/z* 409 ($\text{M}^+ - \text{Me}$), 381, 354 ($\text{M}^+ - \text{C}_5\text{H}_{10}$), 341,

300, 299 (loss of s.c.), 257, and 243. The fragmentation pattern was essentially the same as that of **2b**¹⁾ suggesting that **1b** and **2b** were stereoisomers. The side chain ^1H signals (H-21, H-26, H-27, H-24¹) in the ^1H -NMR spectrum of **1b** were consistent with the corresponding signals for **2b**,²⁾ whereas those due to the skeleton agreed well with those of **1a**. This established the structure of **1b** as (24*S*)-24-methyl-28-norcycloart-25-en-3-one(4-epi-cyclomusalenone).

This is the first unequivocal demonstration of the natural occurrence of 3-oxo-28-norcycloartane-type triterpenes.⁵⁻⁸⁾ The co-occurrence of 28-norcycloartanes **1a** and **1b** with their 4 α -methyl-epimers (**2a**, **2b**) suggests their endogenous formation by isomerization in banana peel tissues.^{9,10)}

Assigned ^{13}C - and ^1H -NMR data of **1a** and **1b** are given in Table 1.

Experimental

Crystallizations were performed from acetone-MeOH. Preparative HPLC was carried out on an octadecyl silica column (Superiorex ODS S-5 μm column, 25 cm $\times$ 10 mm i.d.; Shiseido Co., Ltd., Tokyo) with MeOH (4 ml/min) using an SSC Flow System 3100K (Senshu Scientific Co., Ltd., Tokyo) and an ERC-7520 refractive index detector (ERC Co., Ltd., Tokyo). GLC was run on a Shimadzu GC-14B apparatus using a DB-17 fused silica capillary column (30 m $\times$ 0.3 mm i.d., column temp. 275 $^\circ\text{C}$). In both HPLC and GLC, cholesterol (cholest-5-en-3 β -ol) was the standard for the determination of *R_f* of 3-oxotriterpenes. IR spectra were recorded in KBr with a JASCO FT-IR 300 IR spectrometer. Electron-impact MS and HR-MS were taken on a Hitachi M-80B double focusing gas chromatograph-mass spectrometer (70 eV) using a direct inlet system. NMR spectra were recorded with a JEOL GSX-400 spectrometer at 400 MHz (^1H -NMR) and 100.62 MHz (^{13}C -NMR) in CDCl_3 with tetramethylsilane (TMS) (^1H -NMR) and CDCl_3 at δ 77.0 (^{13}C -NMR) as internal standards, and chemical shifts were recorded in δ values. Banana, which was free of post-harvest agricultural chemicals and imported from Philippines, was purchased at a market in Tokyo.

Isolation Procedure Lyophilized banana peel (300 g) was extracted 3 times on 3 successive days with *n*-hexane at room temperature to give an extract (3.77 g). This was subjected to column chromatography over silica gel (200 g) using the gradient solvent system (*n*-hexane:EtOAc = 1:0—1:4, v/v) to yield a 3-oxotriterpene fraction (644 mg). Preparative HPLC of the fraction yielded **1a** (10 mg), **1b** (2 mg), **2a** (330 mg),²⁾ and **2b** (104 mg).²⁾

4-Epicycloeucalenone [24-Methyl-28-norcycloart-24(24¹)-en-3-one] (**1a**) mp 130—131 $^\circ\text{C}$. *R_f*: 1.06 (HPLC), 2.09 (GLC). IR ν_{max} cm^{-1} : 1720 ($>\text{C}=\text{O}$), 3080, 1640, 887 ($>\text{C}=\text{CH}_2$). MS *m/z* (%): 424 (M^+ ,

11), 409 (4), 381 (5), 340 (5), 327 (5), 326 (5), 325 (3), 300 (7), 299 (13), 297 (3), 257 (3), 245 (3), 243 (2), 231 (3), 229 (3), 219 (5), 55 (100). HR-MS m/z : 424.3700 [Calcd for $C_{30}H_{48}O$ (M^+): 424.3702]; 409.3422 [Calcd for $C_{29}H_{45}O$: 409.3467]; 381.3138 [Calcd for $C_{27}H_{41}O$: 381.3154]; 300.2710 [Calcd for $C_{22}H_{36}$: 300.2814]; 299.2357 [Calcd for $C_{21}H_{31}O$: 299.2373]; 257.1966 [Calcd for $C_{18}H_{25}O$: 257.1904]; 243.1798 [Calcd for $C_{17}H_{23}O$: 243.1748].

4-Epicyclomusalenone [(24S)-24-Methyl-28-norcycloart-25-en-3-one] (1b) mp 125–127 °C. R_{fR} : 1.04 (HPLC), 2.06 (GLC). MS m/z (%): 424 (M^+ , 23), 409 (7), 381 (2), 354 (2), 341 (3), 328 (4), 326 (5), 300 (14), 299 (28), 297 (4), 285 (3), 273 (3), 257 (3), 245 (4), 243 (3), 231 (3), 219 (6), 55 (100). HR-MS m/z : 424.3681 [Calcd for $C_{30}H_{48}O$ (M^+): 424.3702]; 409.3469 [Calcd for $C_{29}H_{45}O$: 409.3467]; 381.3129 [Calcd for $C_{27}H_{41}O$: 381.3154]; 354.2930 [Calcd for $C_{25}H_{38}O$: 354.2921]; 341.2813 [Calcd for $C_{27}H_{37}O$: 341.2842]; 300.2683 [Calcd for $C_{22}H_{36}$: 300.2814]; 299.2345 [Calcd for $C_{21}H_{31}O$: 299.2373]; 257.1972 [Calcd for $C_{18}H_{25}O$: 257.1904]; 243.1782 [Calcd for $C_{17}H_{23}O$: 243.1748].

Acid-Catalyzed Conversion of 4-Epicycloeucaleanone (1a) into Cycloeucaleanone (2a) **1a** (7 mg) was heated under reflux for 1.5 h in 5 ml of EtOH containing 0.2 ml of 20% H_2SO_4 . The solution was poured into water and extracted twice with diethyl ether. Usual work-up of the ether solution gave the reaction mixture, which upon HPLC yielded **2a** (2.6 mg; identified by GLC, HPLC, 1H -NMR, MS), (23*E*)-24-methyl-29-norcycloart-23-en-3-one (**2c**; 0.5 mg) and 24-methyl-29-norcycloart-24-en-3-one (**2d**; 1.1 mg) in addition to the starting material **1a** (0.5 mg).¹¹⁾

(23*E*)-24-Methyl-29-norcycloart-23-en-3-one (2c) Amorphous. R_{fR} : 1.25 (HPLC), 1.89 (GLC). MS m/z (%): 424 (M^+ , 9), 409 (3), 381 (1), 327 (18), 299 (5), 297 (7), 275 (2), 245 (2), 231 (2), 55 (100). HR-MS m/z : 424.3678 [Calcd for $C_{30}H_{48}O$ (M^+): 424.3702]. 1H -NMR: δ 0.40, 0.62 (each 1H, d, $J=4.1$ Hz, H-19), 0.85 (3H, d, $J=6.6$ Hz, H-21), 0.91 (3H, s, H-30), 0.99 (9H, d, $J=6.9$ Hz, H-26, H-27, H-28), 1.01 (3H, s, H-18), 1.56 (3H, s, H-24'), 5.16 (1H, t, $J=7.1$ Hz, H-23).

24-Methyl-29-norcycloart-24-en-3-one (2d) mp 100–103 °C. R_{fR} : 1.35 (HPLC), 2.26 (GLC). MS m/z (%): 424 (M^+ , 19), 409 (5), 381 (2), 341 (10), 328 (4), 299 (11), 297 (5), 257 (4), 245 (3), 55 (100). HR-MS m/z : 424.3681 [Calcd for $C_{30}H_{48}O$ (M^+): 424.3702]. 1H -NMR: δ 0.40, 0.62 (each 1H, d, $J=4.1$ Hz, H-19), 0.91 (3H, s, H-30), 0.92 (3H, d,

$J=6.6$ Hz, H-21), 0.99 (3H, d, $J=6.6$ Hz, H-28), 1.01 (3H, s, H-18), 1.63 (6H, s), 1.64 (3H, s) (H-26, H-27, H-24').

References and Notes

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- 2) Akihisa T., Shimizu N., Tamura T., Matsumoto T., *Lipids*, **21**, 494–497 (1986).
- 3) CAChe with extended MM2 parameters (CAChe Scientific Inc., Beaverton, Oregon, U.S.A.). The conformation with minimum steric energy was obtained from the potential energy map using the "Sequential Search" option. The minimum steric energy calculated was: 178.44 kcal/mol for **1a** and 177.02 kcal/mol for **2a**. Drawings were made using Chem3D software (Cambridge Scientific Computing Inc., Cambridge, Massachusetts, U.S.A.).
- 4) Knapp F. F., Schroepfer G. F., Jr., *Steroids*, **26**, 339–357 (1975).
- 5) The 4 β -methyl assignment of 4-methylsterols from rat skin⁶⁾ has since been shown to be erroneous.⁷⁾ Our reinvestigation of the sterol and 4-methylsterol constituents of both ligulate and tabular flowers of marigold (*Calendula officinalis* L.) did not confirm the presence of the 4 β -methylsterols⁸⁾ (unpublished results).
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- 8) Pyrek J. St., *J. Chem. Soc. (D)*, **1969**, 107–108.
- 9) We have confirmed that isomerization from 4 α -methyl to 4 β -methyl did not take place during our extraction and isolation procedures. Thus, the same extraction and isolation procedures applied to **2a** yielded only unreacted starting material (**2a**). Treatment of 3-oxo-29-norcycloartanes with LiCl in dimethylformamide (DMF) under N_2 (reflux) was reported to afford their 4 β -epimers in low yield (7.5%).¹⁰⁾
- 10) Cattel L., Delprino L., Benveniste P., Rahier A., *J. Am. Oil Chem. Soc.*, **56**, 6–11 (1979).
- 11) This isomerization proceeds *via* keto-enol tautomerism, and our results showed that the equilibrium was shifted to the direction where sterically more stable 4 α -epimer (**2a**) was formed preferentially.