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Stereochemistry of Novel Triterpenes from *Cynanchum hancokianum*

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The absolute stereochemistry of hancokinol, hancolupenone, hancolupenol, hancolupenol hexacosanoate, which are novel triterpenes, from *Cynanchum hancokianum*, has been elucidated by means of spectroscopic [nuclear magnetic resonance (NMR), circular dichroism (CD)] analyses.

Keywords absolute stereochemistry; hancokinol; hancolupenone; hancolupenol; hancolupenol hexacosanoate; triterpene; *Cynanchum hancokianum*; spectroscopic (NMR, CD) analyses

Cynanchum hancokianum (Maxim.) Al. Iljinski. (Asclepiadaceae), distributed in Inner Mongolia, is known as a Chinese folk medicine possessing antitumor activity. We previously obtained two phenanthroindolizine alkaloids, antofine and de-6-*O*-methylantofine, from the hydrochloric acid extract of this plant.[1)] Our further investigations have led to the isolation of four new pentacyclic triterpenes, named hancokinol (**1**),[2)] hancolupenone (**2**),[3)] hancolupenol (**3**)[3)] and hancolupenol hexacosanoate (**4**), from the ethanol extract of the same plant source. Compounds **1**—**4** were obtained on chromatography (silica gel) of the petroleum ether-soluble portion of the ethanol extract, by elution with a mixture of petroleum ether and acetone. This paper deals in detail with the structural elucidation of these compounds by means of spectroscopic (proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR), circular dichroism (CD)) analyses.

Hancokinol (**1**),[2)] $C_{30}H_{50}O$, mp 229—230 °C (MeOH), $[\alpha]_D^{20}$ +16.2° ($CHCl_3$), showed an infrared (IR) hydroxyl band at 3580 cm^{-1}.

Distortionless enhancement by polarization transfer (DEPT) experiments indicated the presence of eight methyls, nine methylenes, seven methines and six quaternary carbons. Protons and carbons were assigned by two-dimensional (2D) NMR spectroscopy, and the parameters are listed in Table I. The position of two *gem*-methyls (C-23, C-24) and four angular methyls (C-25—C-28) were unambiguously determined by the 2D correlations to the surrounding protonated or quaternary carbons. The presence of an isopropyl comprised of two *sec*-methyls at δ_H 0.87 (H_3-29) and 0.90 (H_3-30) and a methine at δ_H 1.49 (H-20) was confirmed by ^{1}H–^{1}H correlation spectroscopy (COSY) experiments. A methine at δ_H 1.57 (H-19) was correlated to a methine at δ_H 1.48 (H-18) and H-20, leading to iso-Pr-19 (^{1}H–^{1}H). A one-proton signal at δ_H 5.54 was attributed to a trisubstituted olefinic proton (H-6) possessing a neighboring methylene at δ_H 2.26 and 2.01 (H_2-7) (^{1}H–^{1}H). ^{1}H–^{1}H and ^{1}H–^{13}C COSY experiments placed a hydroxymethine (δ_H 3.47, δ_C 76.58, C-3) between a methylene (δ_H 1.90, 1.69, δ_C 28.81, C-2) and a quaternary carbon (δ_C 41.32, C-4) possessing two *gem*-methyls (δ_H 1.04, δ_C 27.26, C-23) and (δ_H 1.13, δ_C 25.39, C-24). The hydroxyl was assigned to be axial on the basis of the coupling (t, J=2.5 Hz) observed for H-3. The correlations of two methines at δ_H 1.48 (H-8) and 2.35 (H-10) to neighboring methylenes at δ_H 2.26 (H-7), and 1.59 and 1.42 (H_2-1) were respectively observed in the ^{1}H–^{1}H COSY spectrum.

The electron impact high resolution mass spectrum

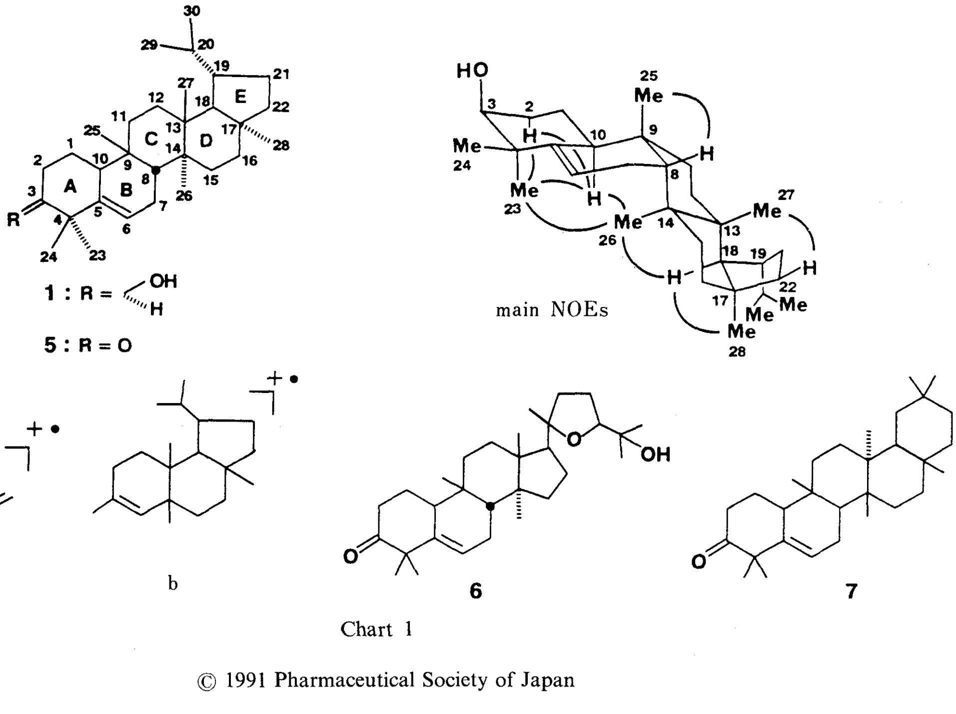


Chart 1

TABLE I. NMR Data for **1**

Carbon No.	Carbon δ_C	Correlated H[a] δ_H	H coupled with C[b]	H coupled with H[c]
1	20.80 t	Hα-1 1.42 m	H_2-2, H-3, H-10	Hβ-1, Hβ-2, H-10
		Hβ-1 1.59 m		Hα-1, H_2-2, H-10
2	28.81 t	Hα-2 1.90 ddt (2.5, 4.0, 14.0)		Hβ-1, Hβ-2, H-3
		Hβ-2 1.69 m		H_2-1, Hα-2, H-3
3	76.58 d	H-3 3.47 t (2.5)	Hβ-1, H_3-23	H_2-2
4	41.32 s		Hβ-2, H_3-23, H_3-24	
5	140.64 s		H_3-23, H_3-24	
6	121.47 d	H-6 5.54 dt (6.0, 2.0)	H_2-7, H-8	H_2-7
7	22.65 t	Hα-7 2.01 dd (19.0, 6.0)	H-8	H-6, Hβ-7
		Hβ-7 2.26 ddd (19.0, 3.2, 2.0)		H-6, Hα-7, H-8
8	44.54 d	H-8 1.48 m	Hβ-7, H_3-25, H_3-26	Hβ-7
9	34.49 s		H-8, H_2-11, H_3-25	
10	37.23 d	H-10 2.35 d (12.0)	H-8, H_3-25	H_2-1
11	30.64 t	H_2-11 1.13 m	H-8, H_3-25	H_2-12
12	30.72 t	Hα-12 1.59 m		H_2-11, Hβ-12
		Hβ-12 1.36 m		H_2-11, Hα-12
13	39.25 s		H-8, H_2-11, Hα-12, H-18, H_3-26, H_3-27	
14	39.62 s		Hβ-7, H-8, H-18, H_3-26, H_3-27	
15	29.38 t	H_2-15 1.25 m	H-8, H_2-16, H_3-26	H_2-16
16	32.65 t	H_2-16 1.49 m	Hβ-22, H_3-28	H_2-15
17	39.92 s		H_2-15, H_2-16, Hb-21, Hβ-22, H_3-28	
18	54.46 d	H-18 1.48 d (2.0)	Hα-12, Hb-21, Hα-22, H_3-27, H_3-28	H-19
19	48.24 d	H-19 1.57 m	Hb-21, Hα-22, H_3-29, H_3-30	H-18, H-20, Ha-21
20	35.96 d	H-20 1.49 m	H-18, H_3-29, H_3-30	H_3-29, H_3-30
21	27.85 t	Ha-21 1.44 m	H-18, H-20, Hβ-22	H-19, Hb-21, Hα-22
		Hb-21 1.67 m		Ha-21, Hβ-22
22	42.44 t	Hα-22 1.25 m	H-18, H_3-28	Ha-21, Hβ-22
		Hβ-22 1.79 dd (11.5, 9.0)		Hb-21, Hα-22
23	27.26 q	H_3-23 1.04 s	H_3-24	
24	25.39 q	H_3-24 1.13 s	H_3-23	
25	28.77 q	H_3-25 0.90 s	Hβ-1, H-8, H-10, H_2-11, H_2-12	
26	15.39 q	H_3-26 0.80 s	H-8, H_2-15	H-8
27	16.78 q	H_3-27 0.88 s	H_2-11, Hβ-12, H-18	
28	33.35 q	H_3-28 0.93 s	H_2-16, H-18, H_2-22	
29[d]	21.32 q	H_3-29 0.87 d (6.8)	H_3-30	H-20
30[d]	23.44 q	H_3-30 0.90 d (6.0)	H_3-29	H-20

a) ^1H–^{13}C (one-bond) COSY. Figures in parentheses are coupling constants (Hz). *b*) ^1H–^{13}C (long-range) COSY. *c*) ^1H–^1H COSY. *d*) These are exchangeable.

TABLE II. NOE Data for **1**

Irradiated H	Observed H (%)
Hβ-1	H_3-24 (1.5), H_3-25 (1.5)
H-3	Hα-2 (5.1), Hβ-2 (4.6), H_3-23 (1.9), H_3-24 (1.3)
Hα-7	H-6 (2.2), Hβ-7 (2.3), H-10 (2.4)
Hβ-7	H-6 (1.7), Hα-7 (6.5), H-8 (2.2)
H-8	H_3-25 (3.4)
H-10	Hα-2 (3.3), H-3 (1.1), Hα-7 (2.3), Hα-12 (4.6), H_3-23 (1.3), H_3-26 (1.1)
Hα-12	H-10 (2.9), Hβ-12 (3.4)
H-18	H_3-26 (1.5), H_3-28 (1.9)
H-19	H_3-29 (1.2)
H-20	H_3-29 (3.4), H_3-30 (1.9)
H_3-23	Hα-2 (5.9), Hα-3 (6.7), H-10 (6.1), H_3-26 (1.0)
H_3-24	H-3 (5.7), H-6 (16.1), H_3-23 (1.1)
H_3-25	Hβ-7 (3.2)
H_3-26	H-6 (2.2), Hα-7 (3.8), H-10 (11.0), H-18 (13.8), H_3-23 (1.1)
H_3-27	Hβ-12 (5.2), Hβ-22 (3.1)
H_3-28	H-18 (20.7), Hα-22 (4.0), H_3-26 (1.0)
H_3-29[a]	H-20 (15.0)
H_3-30[a]	H-20 (30.0)

a) These are exchangeable.

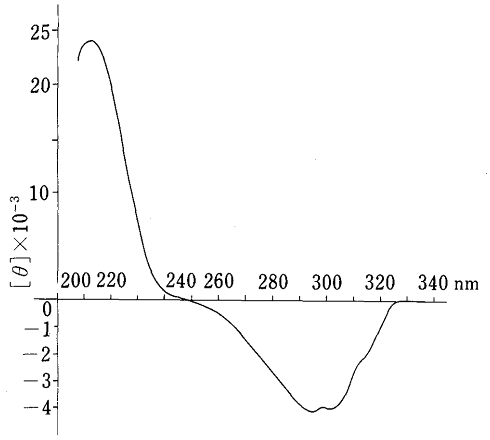


Fig. 1. CD Spectrum of **5**

(EIHRMS) displayed the characteristic fragments at *m/z* 152.1205 (152.1201 for $C_{10}H_{16}O$) (a) and 274.2648 (274.2660 for $C_{20}H_{34}$) (b) arising from a retro-Diels–Alder fission, regarded as suggestive of a Δ^5-compound.[4] Thus, these findings and the NMR data pointed out a 9,13-dimethyl-25,26-dinorlup-5-en-3-ol for **1**.

The nuclear Overhauser effect (NOE) data are given in Table II. The relative stereochemistry was examined assuming a chair form for each ring-A, -C and -D and a half-chair form for ring-B and using a basis of Hα-10. NOEs observed among H-2 (δ_H 1.90), H-10 and H_3-23 suggested their orientations to be axial (α) with respect to ring-A. The mutual enhancements among H-10, H_3-23 and H_3-26 pointed out C(8)-C(14) to be axial (α) and consequently to be H-8 equatorial (β) with respect to ring-B. An NOE observed between H-8 and H_3-25 led to Meβ (ax)-9 (H_3-25) with respect to ring-B, resulting in B/C *cis*. The enhancements linking H-18 to H_3-26 and H_3-28 suggested Meα (ax)-14 (H_3-26), Meα (eq)-17 (H_3-28) and Hα (ax)-18 with respect to ring-D. An NOE observed between Hβ-22 (δ_H 1.79) and H_3-27 displayed Me-13 (H_3-27) and C(17)-C(22) to be axial (β) with respect to ring-D. Thus, C/D *trans* and D/E *cis* were established on the basis of Meβ (ax)-13, Meα (ax)-14, Meα (eq)-17 and Hα (ax)-18. Unfortunately, the orientation of iso-Pr-19 could not be decided by NOE experiments.

A Dreiding model based on the NOE data indicated the presence of a steric repulsion between H_3-23 and H_3-26, so that ring B would deviate from a half-chair form. Actually, an NOE observed between Hα-7 (δ_H 2.01) and H-10 is only explainable by an envelope form with C(9) out of the plane or a half-boat form (probably impossible in practice) for ring-B. The ring-B deformation would be transmitted to ring-A, causing mutual approaches between H-3 and H-10 and between Hβ-1 (δ_H 1.59) and H_3-24. Such steric situations would be responsible for the observed NOEs between them.

An X-ray analysis proved the relative stereochemistry deduced above to be correct.[5] The ring-A and -B deformations, iso-Prα-19 and a ring-E puckered form with C(17) out of the plane were indicated. The ring-E conformation is affected by repulsion between iso-Pr-19 and H_3-28, causing deformation around C(17) in ring-D, and bending of H_3-28 toward H_3-26.[5] If it is assumed that the ring-D and -E solution conformations are similar to the solid-state ones, the mutual approaches between the two methyls account for an NOE observed between them.

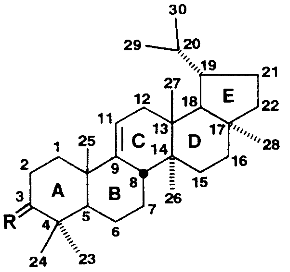
To establish the absolute stereochemistry, we finally examined the CD spectrum of 3-dehydrohancokinol (**5**) which was easily obtained by oxidation of **1** with pyridinium dichromate. 3-Dehydrodesoxogratiogenin (**6**)[6] and alnusenone (**7**)[7] respectively reveal a negative Cotton effect at 295 nm (carbonyl n→π*) in the CD spectra. As depicted in Fig. 1, **5** exhibited a negative Cotton effect at 295 nm and the same curve at 325—249 nm as those of **6** and **7**. On the basis of the structural similarities of ring-A and -B, the 10*S*-configuration was assigned for **5**. Thus, the absolute stereochemistry of **1** was determined to be the 3*S*,8*R*,9*S*,10*S*,13*R*,14*S*,17*S*,18*S*,19*S*-configuration, *i.e.* 9β,13β-dimethyl-25,26-dinor-10α,17α-lup-5-en-3β-ol, on the basis of the relative stereochemistry.

Hancolupenone (**2**),[3] $C_{30}H_{48}O$, colorless plates, mp 241—242 °C (MeOH), $[\alpha]_D^{29}$ +14.4° ($CHCl_3$), showed an IR carbonyl band at 1702 cm^{-1}.

DEPT experiments showed the presence of eight methyls, nine methylenes, six methines and seven quaternary carbons. NMR analysis was carried out in the same manner as employed for **1**. The NMR parameters are given in Table III. ^1H–^1H COSY experiments indicated the presence of an isopropyl comprised to two *sec*-methyls δ_H 0.88 (H_3-29) and 0.90 (H_3-30) and a methine δ_H 1.53 (H-20). The ^1H–^1H and ^1H–^{13}C correlations of a methylene (δ_H 1.76, 1.12, δ_C 38.46, C-22) to a methylene (δ_H 1.76, 1.51, δ_C 28.90, C-21) and a methyl (δ_H 0.92, δ_C 32.60, C-28), which was related to a methine (δ_H 1.55, δ_C 54.17, C-18), suggested iso-Pr-19. A one-proton signal at δ_H 5.31 was attributed to a trisubstituted olefinic proton (H-11) possessing a neighboring methylene at δ_H 1.71 (H_2-12) (^1H–^1H). A carbonyl carbon at δ_C 217.34 (C-3) was correlated to a methylene at δ_H 2.40 (H-2) (^1H–^{13}C, long-range). A methine at δ_H 2.00 (H-8) was correlated to a neighboring methylene at δ_H 1.88 (H-7) (^1H–^1H).

A number of pentacyclic triterpenes with Δ^7, Δ^8 and Δ^9 unsaturations show characteristic mass spectrum (MS) fragments at *m/z* M^+-15 and M^+-167 and almost identical patterns for the remaining minor fragments.[4] As a result, these compounds cannot be distinguished from each other by mass spectroscopy. The electron impact mass spectra (EIMS) of **2** displayed a prominent fragment at *m/z* 206 arising from a retro-Diels–Alder fission in addition to fragments at *m/z* M^+-15 and M^+-167, suggesting a 13-methyl-26-norlup-9(11)-en-3-one for **2** by combining the NMR data.

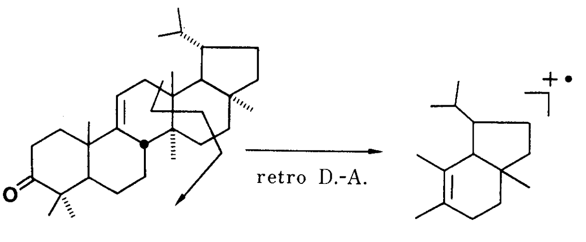
The NOE data are shown in Table IV. The relative stereochemistry was examined assuming a chair form for each ring-A, -B and -D and a half-chair form for ring-C and using a basis of Meβ-10 (H_3-25). NOE experiments led to the following ring fusions: A/B *trans*, Hα (ax)-5 and Meβ (ax)-10 with respect to ring-A and -B; C/D *trans*, Meβ



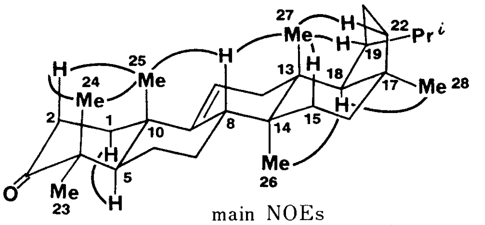
2 : R = O

3 : R = $\overset{\text{OH}}{\underset{\text{H}}{<}}$

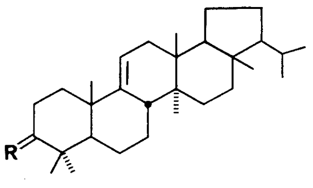
4 : R = $\overset{\text{OCOC}_{25}\text{H}_{51}(n)}{\underset{\text{H}}{<}}$



m/z M^+-167 *m/z* 206

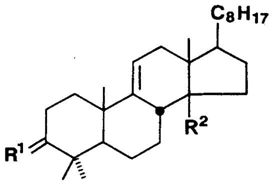


main NOEs



8 : R = O

10 : R = $\overset{\text{OMe}}{\underset{\text{H}}{<}}$



9 : R^1 = O, R^2 = ····Me

11 : R^1 = $\overset{\text{OAc}}{\underset{\text{H}}{<}}$, R^2 = —H

Chart 2

Table III. NMR Data for **2**

Carbon No.	δ_C	Correlated H[a] δ_H	H coupled with C[b]	H coupled with H[c]
1	36.50 t	Hα-1 1.80 dt (5.5, 13.5)	H_3-25	Hβ-1, H_2-2
		Hβ-1 2.05 ddd (13.5, 6.0, 3.0)		Hα-1, H_2-2
2	34.90 t	Hα-2 2.40 ddd (15.5, 5.5, 3.0)		H_2-1, Hβ-2
		Hβ-2 2.72 ddd (15.5, 13.5, 6.0)		H_2-1, Hα-2
3	217.34 s		Hα-2	
4	47.65 s		H-5, H_3-23, H_3-24	
5	53.39 d	H-5 1.36 dd (12.0, 3.0)		H_2-6
6	22.82 t	Hα-6 1.70 m	Hα-7	H-5, Hβ-6, Hβ-7
		Hβ-6 1.55 m		H-5, Hα-6, Hα-7
7	26.72 t	Hα-7 1.14 m	H-5, H_2-6, H-8	Hβ-6, Hβ-7
		Hβ-7 1.88 dq (12.0, 4.0)		Hα-6, Hα-7, H-8
8	42.25 d	H-8 2.00 br d (12.0)	H_3-27	Hβ-7
9	146.78 s		H_3-25	
10	39.18 s		H_3-25	
11	115.04 d	H-11 5.31 t (3.0)	H_2-12	H_2-12
12	36.95 t	H_2-12 1.71 d (3.0)	H_3-27	H-11
13	36.99 s		H-8, H-18, H-19 H_3-26, H_3-27	
14	37.34 s		H_3-26, H_3-27	
15	27.91 t	Hα-15 1.31 m	H_2-16, H_3-26	Hβ-15, H_2-16
		Hβ-15 1.44 m		Hα-15
16	32.23 t	H_2-16 1.45 m	Hα-22, H_3-28	Hα-15
17	41.16 s		H_3-28	
18	54.17 d	H-18 1.55 m	H_3-28	
19	49.53 d	H-19 1.49 m		Hβ-21
20	35.94 d	H-20 1.53 m	H-19, Hα-21	H_3-29, H_3-30
21	28.90 t	Hα-21 1.51 m	H-19, Hβ-22	Hβ-21, H_2-22
		Hβ-21 1.76 m		H-19, Hα-21, Hα-22
22	38.46 t	Hα-22 1.12 m	H-19, Hα-21, H_3-28	H_2-21, Hβ-22
		Hβ-22 1.76 m		Hα-21, Hα-22
23	25.55 q	H_3-23 1.07 s	H_3-24	
24	22.00 q	H_3-24 1.06 s	H-5, H_3-23	
25	21.30 q	H_3-25 1.20 s	Hα-1, H-5	
26	15.95 q	H_3-26 0.75 s		
27	15.67 q	H_3-27 0.74 s	H-18	
28	32.60 q	H_3-28 0.92 s	H_2-16, H-18, H-19, Hα-21	
29[d]	22.32 q	H_3-29 0.88 d (5.8)	H_3-30	H-20
30[d]	23.42 q	H_3-30 0.90 d (5.8)	H_3-29	H-20

a) ^1H–^{13}C (one-bond) COSY. Figures in parentheses are coupling constants (Hz). *b*) ^1H–^{13}C (long-range) COSY. *c*) ^1H–^1H COSY. *d*) These are exchangeable.

(ax)-13 (H_3-27) and Meα (ax)-14 (H_3-26) with respect to ring-C and -D; D/E *cis*, Meα (eq)-17 (H_3-28) and Hα (ax)-18 with respect to ring-D. The enhancements linking to H_3-25 and H_3-27 suggested Hβ (ax)-8 with respect to ring- B and -C. An NOE observed between H-19 and H_3-27 led to iso-Prα-19.

A Dreiding model based on the NOE data showed the presence of steric hindrances in ring-A, -D and -E, probably causing the whole ring deformations. Such steric situations would be responsible for NOEs observed between Hα-1 (δ_H 1.80) and H_3-23, between Hβ-7 (δ_H 1.88) and H_3-25 and between H_3-26 and H_3-28.

An X-ray analysis proved the relative stereochemistry deduced above to be correct.[5] The ring-A, -B and -D deformations were found.

Table IV. NOE Data for **2**

Irradiated H	Observed H (%)
Hα-1	Hβ-1 (8.6), H-5 (2.6)
Hβ-2	Hα-2 (8.6)
Hα-7	Hα-6 (6.9), Hβ-7 (2.6)
Hβ-7	Hα-7 (2.6)
H-8	H_3-25 (1.0)
Hα-15	Hβ-15 (7.8)
Hα-22	Hβ-22 (6.9)
Hβ-22	Hα-22 (6.9)
H_3-23	Hα-1 (3.4), H-5 (10.3), Hα-6 (7.8)
H_3-24	Hβ-2 (2.6), H_3-25 (5.0)
H_3-25	Hβ-2 (3.4), Hβ-7 (5.2), H-8 (4.3), H_3-24 (4.0)
H_3-26	Hα-7 (5.2), H_2-12 (2.2), H-18 (8.6)
H_3-27	H-8 (5.6), Hβ-15 (4.3), H-19 (6.0), Hβ-21, Hβ-22 (5.2)
H_3-28	H_2-16 (2.8), H-18 (2.6), Hα-21 (11.2), H_3-26 (0.4)
H_3-29[a]	H-20 (2.2)
H_3-30[a]	H-18 (1.7), Hα-21 (22.1)

a) These are exchangeable.

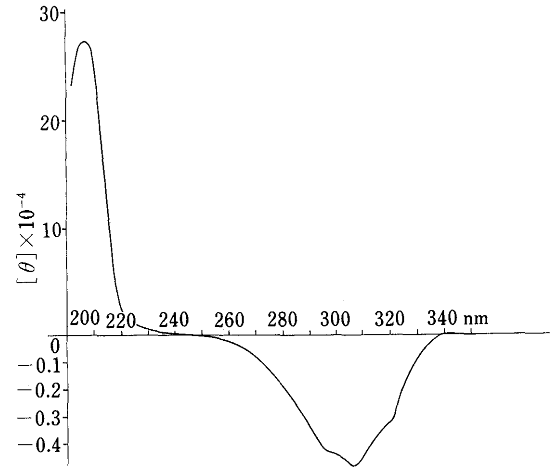


Fig. 2. CD Spectrum of **2**

Arborinone (**8**) and lanost-9(11)-en-3-one (**9**) respectively show a negative Cotton effect in the carbonyl n→π* region in the optical rotatory dispersion (ORD) spectra.[8] Cylindrin (**10**), 4,4-dimethyl-5α,14β-cholest-9(11)-en-3β-ol acetate (**11**), **8** and analogous $\Delta^{9(11)}$-steroids respectively exhibit a positive Cotton effect in the olefinic π→π* region in the CD spectra.[9] On the other hand, arundoin (**10**: 8α,13α,14β,17α,21α) indicates a negative Cotton effect.[9] A negative CD Cotton effect at 306 nm and a positive one at 201 nm observed for **2** (Fig. 2) led to the 10*S*- and 8*S*, 10*S*-configurations for **2**, respectively. Thus, the absolute stereochemistry was determined to be the 5*R*,8*S*,10*S*,13*R*,14*S*,17*S*,18*S*,19*S*-configuration, *i.e.* 13β-methyl-26-nor-17α-lup-9(11)-en-3-one.

Hancolupenol (**3**),[3] $C_{30}H_{50}O$, colorless plates, mp 215—217 °C (MeOH), $[\alpha]_D^{29}$ +14.9° ($CHCl_3$), showed an IR hydroxyl band at 3602 cm^{-1}.

As shown in Table V,[10] NMR spectroscopy revealed that **2** and **3** possess the same framework with a difference in the 3-substituent. Sodium borohydride reduction of **2** gave **3**. Couplings (dd, J = 11.5, 4.0 Hz) observed for H-3 indicated HOβ (eq)-3, *i.e.* the 3*S*-configuration. The CD spectrum of **3** showed a positive Cotton effect at 203 nm, being in accord with the absolute stereochemistry of **2**.

Table V. NMR Data for **3**

Carbon		Correlated H[a] δ_H	H coupled with H[b]
No.	δ_C		
1	35.86 t	Hα-1 1.72 m Hβ-1 1.62 ddd (11.0, 4.5, 2.5)	Hβ-1 Hα-1
2	27.68 t	Hα-2 1.14 m Hβ-2 1.69 m	Hβ-2 Hα-2, H-3
3	78.96 d	H-3 3.22 dd (11.5, 4.0)	Hβ-2
4	39.06 s		
5	52.51 d	H-5 0.88 m	H_2-6
6	21.66 t	Hα-6 1.69 m Hβ-6 1.44 m	H-5, Hβ-6 H-5, Hα-6
7	27.14 t	Hα-7 1.12 m Hβ-7 1.85 ddd (12.5, 8.0, 3.5)	H_2-6, Hβ-7, H-8 H-6, Hα-7, H-8
8	42.17 d	H-8 1.94 br d (12.5)	H_2-7
9	148.25 s		
10	39.50 s		
11	113.71 d	H-11 5.25 dd (6.0, 3.0)	H_2-12
12	36.92 t	H_2-12 1.68 m	H-11
13	37.01 s		
14	37.31 s		
15	27.91 t	Hα-15 1.29 m Hβ-15 1.38 m	Hβ-15, H_2-16 Hα-15
16	32.65 t	H_2-16 1.44 m	Hα-15
17	41.19 s		
18	54.22 d	H-18 1.53 m	
19	49.53 d	H-19 1.50 m	Hβ-21
20	35.97 d	H-20 1.55 m	H_3-29, H_3-30
21	28.92 t	Hα-21 1.51 m Hβ-21 1.74 m	Hβ-21 H-19, Hα-21
22	38.50 t	Hα-22 1.16 m Hβ-22 1.74 m	Hβ-21, Hβ-22 Hα-22
23	28.18 q	H_3-23 0.98 s	
24	15.61 q	H_3-24 0.81 s	
25	21.83 q	H_3-25 1.01 s	
26	15.67 q	H_3-26 0.71 s	
27	16.01 q	H_3-27 0.74 s	
28	32.62 q	H_3-28 0.92 s	
29[c]	22.33 q	H_3-29 0.88 d (6.0)	H-20
30[c]	23.42 q	H_3-30 0.90 d (6.0)	H-20

a) ^1H–^{13}C (one-bond) COSY. Figures in parentheses are coupling constants (Hz). *b)* ^1H–^1H COSY. *c)* These are exchangeable.

Hancolupenol hexacosanoate (**4**), $C_{56}H_{100}O_2$, colorless needles, mp 99—101 °C ($CHCl_3$), $[\alpha]_D^{29}$ +18.0° ($CHCl_3$), showed an IR carboxyl band at 1720 cm^{-1}. ^{13}C-NMR spectroscopy indicated the presence of one methyl, a number of methylenes and one carboxyl carbon in addition to the carbon signals corresponding to **3**, suggesting **4** to be a fatty acid ester of **3** (H-3, δ_H 4.83). Hydrolysis of **4** gave **3** and hexacosanoic acid (**12**), each of which was identified with an authentic sample by direct comparison.

Experimental

General Experimental Procedures Melting points (uncorr.) were determined on a micro hot-stage apparatus. Specific rotations were taken on a JASCO DPI-181 polarimeter. Spectra were recorded on the following spectrometers: IR, Hitachi 260-30; ultraviolet (UV), Hitachi EPS-2U; CD, JASCO J-20; ^1H-NMR, Varian XL-400 at 400 MHz (reference, tetramethylsilane (TMS)); ^{13}C-NMR, Varian XL-400 at 100.6 MHz (reference, TMS); EIMS, Field desorption (FD) MS and fast atom bombardment (FAB) MS, JEOL JMS DX-300; elemental analysis, Perkin-Elmer 240B.

All NMR spectra were taken at a probe temperature of 20 °C in $CDCl_3$ using a 5 mm tube. The measuring conditions employed for **1**—**3** are shown. The values in parentheses are referred to **2** and **3**, being different from those of **1**.

The DEPT spectra were recorded using the $\theta = 90°$ and 142° pulses to separate the CH/CH_3 and CH_2 lines phased "up and down," respectively. Acquisition data were number of scans, 128—32000 (5120—32000, 25600—32000); relaxation delay for protons, 2 s; and 90° pulse widths, 31.0 μs and 9.6 μs for ^1H and ^{13}C, respectively. The delay between pulses (3.57 ms) was set to $1/2J$(CH), where J(CH) was taken to be 140 Hz.

^1H–^1H COSY was done with a ^1H single probe; relaxation time 1 s; 90° (^1H) = 14.3 μs; 90° mixing pulse; $F_1 = F_2 = 712$ (1038, 2106) Hz; data matrix 512 × 64 (1024 × 128 for **3**): 126 (156, 64) scans during 64 (128 for **3**) time increments (zero filling in F_1); 2 dummy scans; spectra were symmetrized about a diagonal axis using the FOLDT command after 2D transformations.

^1H–^{13}C COSY was done under the following conditions: ^{13}C, 30—105 MHz probe; relaxation time, 1 s; 1 dummy scan; 90° ^1H and ^{13}C pulses were calibrated at 31.0 and 9.6 μs, respectively. One-bond correlation: $F_1 = 702$ (2048, 1907) Hz, $F_2 = 6618$ (10616, 10788) Hz; data matrix 1024 × 24 (2048 × 48 for **2** and **3**); 320 (704, 2560) scans during 64 (48 for **2** and **3**) time increments; acquisition time 0.077 (0.096, 0.095) s; $^1J_{CH}$ (average) 140 Hz; size of final data points, 2000. Long-range correlation: $F_1 = 2071$ (2048) Hz, $F_2 = 13351$ (13793) Hz; data matrix 2048 × 64; 704 (3712) scans during 64 (48) time increments (zero filling in F_1); 1 dummy scan; acquisition time 0.077 (0.074) s; $^{LR}J_{CH}$ (average) 7.0 Hz; size of final data points 2000.

The NOE spectra were recorded by means of NOE difference spectroscopy. The pre-irradiation time of each resonance was 2.25 s. The interpulse delay was 0 s. The irradiation data sets were interleaved to cancel drift and changing magnet homogeneity. These spectra were transformed by the difference between two free induction decays.

Extraction and Isolation The roots of *C. hancokianum* were collected in Inner Mongolia. Plant material was identified by Prof. Y. Guo, Shenyang College of Pharmacy, and a specimen is deposited in the Herbarium of Shenyang College of Pharmacy.

The air-dried, powdered plant material (15 kg) was extracted with boiling EtOH (30 l × 4) for several hours. MeOH–H_2O (6 : 4) (2.5 l) was added to the EtOH extract (2.3 kg), and the whole was extracted with petroleum ether (1.4 l × 5). A part (80%) of the whole petroleum ether extract (250 g) was chromatographed over silica gel (1.2 kg); elution was conducted successively with a mixture of petroleum ether and Me_2CO in 95 : 5 (solvent I), 90 : 10 (solvent II) and 85 : 15 ratio (solvent III). The product (2.2 g) from the solvent I eluate (1.5 l) was rechromatographed over silica gel (96 g) with petroleum ether–Me_2CO (97 : 3) (3 l) to yield **4** (90 mg). Further, the solvent I eluate (6.5 l) afforded **2** (150 mg). The solvent II eluate (1.5 l) gave **1** (1.3 g). Subsequent elution (1.5 l) yielded **3** (120 mg) on rechromatography of the crude eluate (2.8 g) over silica gel (100 g) eluting with solvent I (3.5 l). β-Sitosterol (2.4 g) was obtained by elution with solvent III (1 l). β-Sitosterol was identified with an authentic sample by direct comparison.

Hancokinol (1) Colorless needles, mp 229—230 °C (MeOH), *Rf* 0.45 (silica gel, $CHCl_3$). $[\alpha]_D^{20}$ +16.2° ($c = 0.77$, $CHCl_3$), +16.6° ($c = 0.49$, dioxane). IR ν ($CHCl_3$) cm^{-1}: 3580, 2936, 2866, 1449, 1376, 1136, 1106, 978. UV $\lambda_{max}^{dioxane}$ nm (ε): 278 (315). CD ($c = 8.7 \times 10^{-4}$, dioxane) $[\theta]^{20}$ (nm): 0 (228), +43100 (207) (positive maximum). EIHRMS *m/z*: M$^+$, 426.3862 (426.3862 for $C_{30}H_{50}O$). *Anal.* Calcd for $C_{30}H_{50}O$: C, 84.40; H, 11.81. Found: C, 84.17; H, 11.71.

3-Dehydrohancokinol (5) A mixture of **1** (5.0 mg) and pyridinium dichromate (31.0 mg) in dimethylformamide (0.06 ml) was stirred at room temperature overnight. The reaction mixture was diluted with H_2O and extracted with ether (5 ml × 3). Work-up of the organic layer, followed by preparative thin-layer chromatography (TLC) (silica gel, C_6H_6) of the product, gave **5** (4.5 mg, 90.5%) as colorless needles, mp 115—117 °C (MeOH), *Rf* 0.48 (silica gel, C_6H_6), $[\alpha]_D^{20}$ +17.2° ($c = 0.50$, $CHCl_3$), +17.6° ($c = 0.50$, dioxane). IR ν ($CHCl_3$) cm^{-1}: 2928, 2863, 1702, 1450, 1378, 1143, 1123, 1103, 963. UV $\lambda_{max}^{dioxane}$ (ε): 354 (257) (sh), 158 (300) (sh). CD ($c = 8.4 \times 10^{-4}$, dioxane) $[\theta]^{20}$ (nm): 0 (325), −2120 (314) (sh), −3990 (303) (negative maximum), −3940 (300) (positive maximum), −4100 (295) (negative maximum), −2120 (275) (sh), 0 (249), +24200 (212) (positive maximum). ^{13}C-NMR δ: 25.59 (t, C-1), 38.60 (t, C-2), 215.27 (s, C-3), 50.83 (s, C-4), 142.12 (s, C-5), 120.28 (d, C-6), 22.80 (t, C-7), 44.28 (d, C-8), 34.94 (s, C-9), 38.08 (d, C-10), 30.58 (t, C-11), 30.63 (t, C-12), 39.23 (s, C-13), 39.79 (s, C-14), 29.42 (t, C-15), 32.62 (t, C-16), 39.93 (s, C-17), 54.49 (d, C-18), 48.25 (d, C-19), 35.94 (d, C-20), 27.84 (t, C-21), 42.54 (t, C-22), 27.98 (q, C-23), 22.54 (q, C-24), 28.66 (q, C-25), 15.24 (q, C-26), 16.86 (q, C-27), 33.38 (q, C-28), 21.27 (q, C-29), 23.43 (q, C-30). EIHRMS *m/z*: M^+, 424.3704 (424.3704 for $C_{30}H_{48}O$). *Anal.* Calcd for $C_{30}H_{48}O$: C, 84.84; H, 11.39. Found: C, 84.63; H, 11.38.

Hancolupenone (2) Colorless plates, mp 241—242 °C (MeOH), $[\alpha]_D^{29}$ $+14.4°$ ($c=0.20$, $CHCl_3$), $+16.7°$ ($c=0.20$, hexane). IR ν ($CHCl_3$) cm^{-1}: 2935, 2875, 1702, 1605, 1465, 1375. CD $[\theta]^{28}$ (nm): 0 (339), -328 (319) (sh), -480 (306) (negative maximum), -433 (296) (sh), 0 (245) ($c=1.45\times10^{-3}$, hexane), $+27400$ (207) (positive maximum) ($c=8.82\times10^{-4}$, hexane). EIHRMS *m/z*: M^+, 424.3698 (424.3707 for $C_{30}H_{48}O$). *Anal.* Calcd for $C_{30}H_{48}O$: C, 84.84; H, 11.39. Found: C, 84.67; H, 11.45.

Hancolupenol (3) Colorless plates, mp 215—217 °C (MeOH), $[\alpha]_D^{29}$ $+14.9°$ ($c=0.40$, $CHCl_3$), $+14.4°$ ($c=0.40$, hexane). IR ν ($CHCl_3$) cm^{-1}: 3602, 2927, 2909, 1599, 1457, 1374. CD ($c=1.78\times10^{-3}$, hexane) $[\theta]^{28}$ (nm): 0 (221), $+27310$ (203) (positive maximum). EIHRMS Calcd for $C_{30}H_{50}O$: M, 426.3860. Found *m/z*: M^+, 426.3846.

Reduction of 2 to 3 A solution of **2** (8.2 mg) and $NaBH_4$ (0.5 mg) in MeOH–tetrahydrofuran (1 : 1) (3 ml) was stirred at room temperature for 30 min. The reaction mixture was concentrated *in vacuo*, and the residue was extracted with ether (5 ml × 3). Work-up of the organic layer gave **3** (7.4 mg, 90%) as colorless plates, mp 215—217 °C (MeOH), which was identified with natural hancolupenol by direct comparison.

Hancolupenol Hexacosanoate (4) Colorless needles, mp 99—101 °C ($CHCl_3$), $[\alpha]_D^{29}$ $+18.0°$ ($c=0.10$, $CHCl_3$). IR ν ($CHCl_3$) cm^{-1}: 2931, 2866, 1720, 1606, 1466, 1378. ^{13}C-NMR δ: 35.54 (t, C-1), 31.92 (t, C-2), 80.56 (d, C-3), 38.02 (s, C-4), 52.96 (d, C-5), 21.53 (t, C-6), 27.04 (t, C-7), 42.16 (d, C-8), 147.87 (s, C-9), 39.37 (s, C-10), 113.93 (d, C-11), 36.93 (t, C-12), 37.01 (s, C-13), 37.33 (s, C-14), 27.92 (t, C-15), 32.29 (t, C-16), 41.19 (s, C-17), 54.22 (d, C-18), 49.54 (d, C-19), 35.97 (d, C-20), 28.92 (t, C-21), 38.50 (t, C-22), 28.15 (q, C-23), 14.11 (q, C-24), 21.86 (q, C-25), 15.68 (q, C-26), 16.01 (q, C-27), 32.62 (q, C-28), 22.33 (q, C-29), 23.42 (q, C-30), 173.69 (s, CO), 34.86, 29.59, 25.17, 24.22, 22.69 (each t, CH_2), 29.70 (t, $CH_2\times n$), 16.83 (q, Me). ^1H-NMR δ: 4.83 (dd, $J=12.0$, 4.5 Hz, H-3), 2.30 (t, $J=7.5$ Hz, CH_2CO_2). FAB and FDMS *m/z*: M^++1, 805 (804 for $C_{56}H_{100}O_2$).

Hydrolysis of 4 A mixture of **4** (2.7 mg), 2.5% KOH/MeOH (0.3 ml) and C_6H_6 (0.4 ml) was stirred at room temperature for 4 h. The reaction mixture was concentrated *in vacuo*, and the residue was extracted with C_6H_6 (2 ml × 2). Work-up of the organic layer afforded **3** (1.7 mg) as colorless plates, mp 215—217 °C (MeOH), which was identified with natural hancolupenol by direct comparison. The C_6H_6-insoluble portion was acidified with 5% HCl and evaporated to dryness, then extracted with AcOEt (2 ml × 2). Work-up of the organic layer gave **12** (1.0 mg) as colorless plates, mp 84—86 °C (AcOEt). EIHRMS Calcd for $C_{26}H_{52}O_2$: M, 396.3967. Found *m/z*: M^+, 396.3968. Compound **12** was identified with an authentic sample of haxacosanoic acid by direct comparison.

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