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COMPOSITE CONSTITUENTS: THREE GAMMACER-16-ENE DERIVATIVES, NOVEL TRITERPENOIDS ISOLATED FROM ROOTS OF *PICRIS HIERACIOIDES* SUBSP. *JAPONICA*

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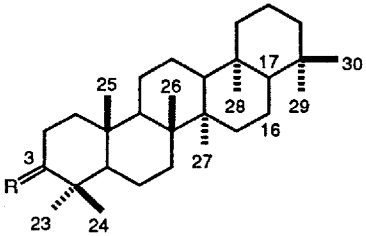
SUMMARY: New triterpenoids, gammacer-16-en-3β-yl acetate (**3**), gammacer-16-en-3β-ol (**4**) and gammacer-16-en-3α-ol (**5**) were isolated from the fresh roots of *Picris hieracioides* subsp. *japonica*, Compositae. Their structures were established by chemical and spectral methods. X-ray crystallographic study of the corresponding ketone (**6**) confirmed the structures of **3**, **4** and **5**.

The rare gammacerane triterpenoid, tetrahymanol (**1**) was first reported from the protozoan *Tetrahymena pyriformis*[1] and secondly from the fern, *Oleandra wallichii* (HOOK.) PR.[2] Recently **1** and tetrahymanyl acetate (**2**) were found in some of the Polypodiaceous[3] and Pteridaceous[4] ferns in our Laboratory.

In the course of the study on Composite triterpenoids,[5, 6, 7] we examined the latex components of the fresh roots of *Picris hieracioides* LINNÉ. subsp. *japonica*. (THUNB.) KLYROV. (Kozorina in Japanese, Compositae). From the n-hexane extract of the roots, we isolated three novel gammacerane triterpenoids, gammacer-16-en-3β-yl acetate (**3**, estimated yield, 0.014% of the dried plant materials), gammacer-16-en-3β-ol (**4**, in a small experimental yield) and gammacer-16-en-3α-ol (**5**, 0.008%), as the first example of a naturally occurring gammacerane triterpenoid with a double bond in the molecule, together with germanicyl acetate, β-amyrin acetate, α-amyrin acetate, bauerenyl acetate, isobauerenyl acetate, taraxasteryl acetate, ψ-taraxasteryl acetate and lupenyl acetate, and some of their corresponding alcohols. In this paper, we wish to report the isolation and structure elucidation of compounds **3**, **4** and **5**.

The n-hexane extract from 8.89 kg of the fresh roots, collected at Inagi-city in Tokyo in June,



1		R=αH, βOH
2		R=αH, βOAc
3	Δ^{16},	R=αH, βOAc
4	Δ^{16},	R=αH, βOH
5	Δ^{16},	R=αH, βOH
6	Δ^{16},	R=O

was chromatographed on Si Gel to give an acetate fraction (10.0 g, an estimated yield of 0.48% of the dried materials) as a n-hexane-benzene (8:2) eluant and two alcohol fractions (axial and equatorial alcohols) as a benzene eluant. The former fraction was chromatographed repeatedly on 20%-$AgNO_3$-Si Gel followed by HPLC (C-18 reverse phase, CH_3CN) to give **3**, mp 287-288°C, $[\alpha]_D^{23}$+36.0° ($CHCl_3$, c=0.5), Rt_R 5.22, M^+ m/z 468.3948 ($C_{32}H_{52}O_2$), IR ν_{max}^{KBr} cm^{-1}: 1730, 1250. The axial alcohol fraction was chromatographed on Si Gel followed by recrystallization from acetone to give **5**, mp 269-270 °C, $[\alpha]_D^{23}$+9.9° ($CHCl_3$, c=0.6), Rt_R 4.03, M^+ m/z 426.3881 ($C_{30}H_{50}O$), IR ν_{max}^{KBr} cm^{-1}: 3510, 1060. After acetylating the equatorial alcohol fraction with acetic anhydride/pyridine, this acetate mixture was purified by the same way as shown above to afford **3**. Hydrolysis of **3** with 5%-KOH gave gammacer-16-en-3β-ol (**4**), mp 254-256°C, $[\alpha]_D^{23}$ +36.6° ($CHCl_3$, c=0.3).

Table 1. ^1H-Chemical Shifts (δ) in $CDCl_3$ Solution on JEOL GX 270 at 270 MHz

	Methyl proton signals								Other protons	
	H-23	H-24	H-25	H-26	H-27	H-28	H-29	H-30	H-3	H-16
1	0.968	0.759	0.811	0.951	0.960	0.811	0.788	0.843	3.194dd (5.5, 10.6) [α]	
2	0.840	0.834	0.840	0.947	0.960	0.810	0.790	0.840	4.476dd (5.7, 10.4) [α]	
3	0.862	0.845	0.862	0.951	0.980	1.056	1.078	1.125	4.488dd (6.0, 10.4) [α]	5.439dd (3.1, 4.8)
4	0.980	0.766	0.839	0.947	0.980	1.055	1.073	1.122	3.206dd (5.5, 10.9) [α]	5.442dd (3.1, 4.8)
5	0.949	0.832	0.854	0.949	0.997	1.055	1.075	1.123	3.399dd (2.2, 2.2) [β]	5.443dd (3.1, 4.8)
6	1.084	1.029	0.937	0.988	0.988	1.060	1.084	1.126		5.449dd (3.1, 4.8)

Coupling constant are shown in parentheses and acetyl methyl protons were observed at: δ 2.042 in **2**, δ 2.044 in **3**.

Table 2. ^{13}C-Chemical Shifts (δ) in $CDCl_3$ Solution on JEOL GX 270 at 68 MHz

	Carbon numbers														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	38.7	27.3	79.0	38.8	55.2	18.7	33.1	41.9	50.2	37.1	21.1	21.3	50.3	41.7	33.1
2	38.5	23.8	81.0	37.9	55.4	18.4	33.4	41.9	50.3	37.1	21.4	21.2	50.4	42.0	33.2
3	38.4	23.7	81.0	37.8	55.3	18.7	33.4	41.3	50.3	37.0	21.4	22.6	46.5	39.4	33.5
4	38.7	27.4	79.1	38.9	55.3	18.7	33.5	41.3	50.5	37.1	21.4	22.7	46.5	39.4	33.4
5	33.3	25.4	76.3	37.5	49.0	18.7	33.4	41.5	50.2	37.2	21.2	22.7	46.5	39.5	33.4

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	18.7	56.3	37.3	40.3	18.7	42.1	33.2	28.0	15.4	15.9	16.5	15.9	15.9	21.5	33.4
2	18.8	56.3	37.4	40.5	18.8	42.3	33.3	28.0	16.6	16.0	16.6	16.6	16.0	21.6	33.3
3	117.8	147.7	37.7	41.5	18.1	41.8	36.1	28.1	16.6	16.2	16.9	17.5	29.9	20.6	33.5
4	117.8	147.7	37.7	41.5	18.2	41.8	36.1	28.1	15.5	16.2	16.9	17.6	29.9	20.6	33.5
5	117.9	147.7	37.6	41.5	18.1	41.8	36.1	28.3	22.2	16.0	16.9	17.7	29.9	20.7	33.5

Acetyl signals were observed at: δ 21.3, 170.9 in **2**, δ 21.3 ,171.0 in **3**.

The ^1H-NMR spectrum (Table 1) of **3** showed eight tertiary methyl groups, a trisubstituted double bond and a 3β acetoxyl group. The electron impact low resolution mass spectrum of **3** (Chart 1) showed the base peak at m/z 189 (a) and the other major peaks at m/z (rel. int.): 204 (34, b), 203 (33, c), 187 (97, d), 150 (38, e) and 135 (56, f). This fragmentation pattern has not been observed in the known triterpenoid ring systems, and a new system having Δ^{16} double bond was suggested. The latter two fragments (e and f) were resulted by an allylic cleavage in ring D. The ^{1}H-chemical shifts of methyl groups attached to the A, B and C rings (C-23—C-26) in **3** were slightly different from those of **2**, but the fragment, m/z 189 (base peak in **2**) indicate that the A, B and C rings of **3** could be the same as those of **2**. This identity of the left counterpart of **3** with that of **2** was also clearly indicated by comparison of their ^{13}C-NMR (Table 2). The above evidences strongly suggested that the structure of **3** is gammacer-16-en-3β-yl acetate.

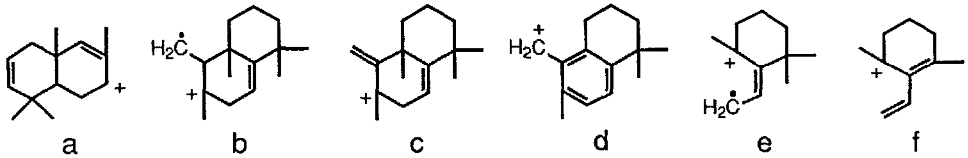


Chart 1

The mass spectrum of **5** showed the same fragments [m/z 204 (20), 203 (24), 189 (100), 187 (45), 150 (21) and 135 (48)] as those of **3**. The olefinic proton signal of compound **5** showed the same splitting pattern and chemical shift as those of **3**, and the chemical shifts of the methyl signals (C-26, 28, 29 and 30) except for C-27 were very similar to those of **3** in the ^{1}H-NMR. Also the ^{1}H-NMR of **5** showed the presence of an axial hydroxyl group at C-3 (Table 1). Thus **5** was suggested to be gammacer-16-en-3α-ol.

Gammacer-16-en-3β-ol (**4**), hydroysis product of **3** with 5%-KOH, was oxidized with CrO_3-pyridine complex to give gammacer-16-en-3-one (**6**), mp 259-261°C, $[\alpha]_D^{23}$ +58.3° ($CHCl_3$, c=0.7). This compound was also obtained from **5** in the same manner. Therefore the structural relationship among **3**, **4**, **5** and **6** was established.

To confirm the structures of **3**, **4** and **5** unequivocally, a single crystal X-ray diffraction studies of **6** was carried out. The crystals of **6**, colorless prism, grown from acetone was mounted on a Rigaku automatic four-circle diffractometer equipped with a graphite monochrometer. *Crystal data*: gammacer-16-en-3-one; $C_{30}H_{48}O$: MW=424.7, monoclinic; a=7.520 (3), b=10.725 (5), c=15.804 (8) Å, β=104.12 (4)°, V=1235.9 (10) Å^3, D_X=1.1413 g.cm^{-3}, Z=2, space group $P2_1$. Reflections were scanned up to Θ=130° using Cu-Kα radiation. Of the 2355 reflections measured, 2237 having $I \geqq 2\sigma(I)$ were used for the following structure determination and refinements. The structure was solved by the direct method (MULTAN program), and refined by the block-diagonal least squares method with anisotropic temperature factors for nonhydrogen atoms and isotropic ones for hydrogen atoms to R factor of 0.05. An ORTEP drawing of the molecular structure of **6** is given in Fig 1.[8]

The isolation of these gammacerane triterpenoids having a double bond in the molecule is the first example from a natural source. Biogenetically, cyclization of squalene or squalene oxide lead to the common cation shown in Chart 2 and then the hopane and isohopane cations and two types of the gammacerane cations. Gammacer-16-ene derivatives are very important from the

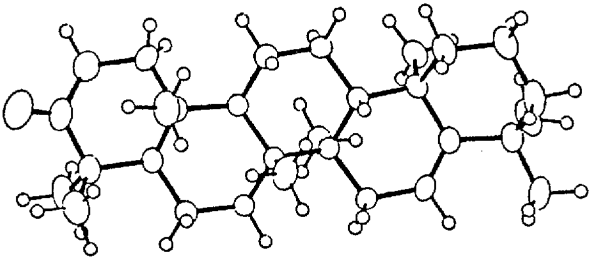


Fig. 1 An ORTEP Drawing of the Molecule Structure of Gammacer-16-en-3–one (**6**)

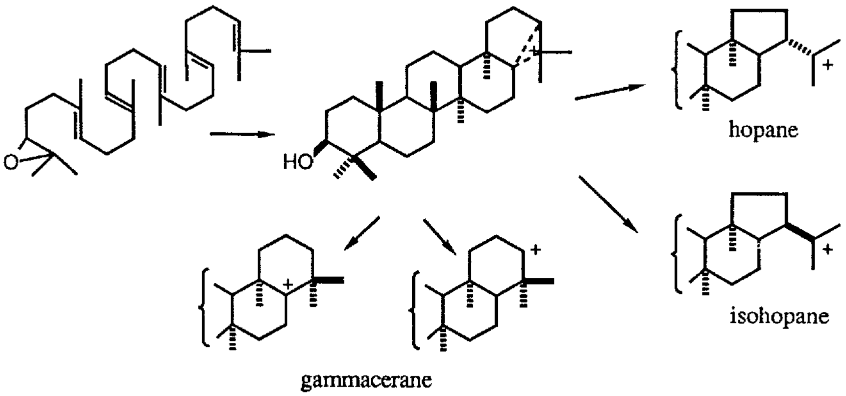


Chart 2

biogenetic point of view, because a report of new groups of the migrated gammacerane triterpenoids will appear very soon.

REFERENCES AND NOTES

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4) Unpublished data, **1** was isolated from following Pteridaceous ferns: *Adiantum monochlamys* EATON, *A. pedatum* LINNÉ, *A. caudatum* LINNÉ, *A. flabellulatum* LINNÉ in our laboratory.
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8) Final crystallographic coordinates and a table of bond distances have been deposited at the Cambridge Crystallographic Data Centre.

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