NOTE

Widespread Occurrence of Cucurbita-5, 24-dienol in Cucurbitaceae

Toshihiro AKIHISA*, P. GHOSH**, Swapnadip THAKUR**,
Fumiko U. ROSENSTEIN***, Toshitake TAMURA*,
and Taro MATSUMOTO*

* College of Science and Technology, Nihon University (1-8, Kanda Surugadai, Chiyoda-ku, Tokyo 〒101)

** Department of Chemistry, University of Burdwan (Burdwan, 713104, India)

*** Department of Nutrition and Food Science, College of Agriculture, The University of Arizona
(Tucson, Arizona, 85721, U.S.A.)

The extracted lipids of 28 seed and mature plant (leaves and stems, pericarp of the fruit, and roots) materials from 11 genera of the family Cucurbitaceae were demonstrated to contain cucurbita-5, 24-dienol, a cucurbitane type Δ^5 -unsaturated 3β -monohydroxy triterpene and present with it in some cases were two Δ^5 -unsaturated pentacyclic 3β -monohydroxy triterpenes, glutinol and simiarenol. Cucurbita-5, 24-dienol is considered to be an important intermediate in the biogenesis of cucurbitacins found in Cucurbitaceae.

1 Introduction

A number of plants of the family Cucurbitaceae contain cucurbitacins^{1),2)}, an important group of highly oxygenated tetracyclic triterpenoids having a cucurbitane skeleton and a wide range of biological activities^{1),3),4)}. We have recently shown the occurrence of 10 α -cucurbita-5, 24-dien-3 β -ol (cucurbita-5, 24-dienol) (1), which represents the parent compound of cucurbitacins, in the seeds of some Cucurbitaceae including *Lagenaria leucantha* var. *Gourda* (gourd)⁵⁾. More recently, it has been demonstrated that the triterpene (1) was a precursor in the biosynthesis of cucurbitacin-C in the seedlings of *Cucumis sativus*⁶⁾ which prompted us to examine the occurrence of (1) in the plant materials of a further species of the family Cucurbitaceae. This paper described the demonstration of widespread occurrence of (1) in the seeds and mature plants of Cucurbitaceae, which was, in some cases, accompanied with further two Δ^5 -unsaturated pentacyclic 3β -monohydroxy triterpenes, *i.e.*, D : B-friedo-olean-5-en-3 β -ol (10 α -glut-5-en-3 β -ol; glutinol) (2) and E : B-friedo-hop-5-en-3 β -ol (10 α -adian-5-en-3 β -ol; simiarenol) (3).

2 Experimental

2.1 General Methods

Crystallizations were performed in acetone-methanol (MeOH). Melting points (mp) taken on a heated block were uncorrected. Unsataponifiable lipid was obtained from a lipid by saponification with 5% KOH in MeOH under reflux followed by extraction with isopropyl ether. Acetylation was performed in acetic anhydride-pyridine at room temp. overnight. Preparative thin-layer chromatography (TLC) on silica gel (20 $\times$ 20 cm, 0.5 mm thick) was developed 3 times using hexane-ethyl acetate (6 : 1). Gas liquid chromatography (GLC) was performed on an OV-17 glass capillary column (30 m $\times$ 0.3 mm i.d., column temp. 260°C) under the conditions already described⁷⁾. The relative retention time (RRT) values were taken relative to cholesteryl acetate. Mass spectra (EI-MS, 70 eV) were taken on a gas chromatograph-mass spectrometer (GC-MS) equipped with a glass column (2 m $\times$ 3 mm i.d., column temp. 270°C) packed with a 2% OV-17 on Gas Chrom-Z. Proton nuclear magnetic resonance (¹H NMR) spectra (100 MHz) were recorded in a CDCl₃ solution, with tetramethylsilane as an internal standard.

2.2 Materials, Extraction and Separation of Triterpene Alcohol Fraction

Twenty-eight seed and mature plant materials obtained from 22 species of 11 genera of the family Cucurbitaceae used in this study are shown in Table-1. The sources of the plant materials were described in our previous article⁹⁾. Cucurbita-5,24-dienol (1)⁵⁾, glutinol (2)⁷⁾, and simiarenol (3)⁷⁾ were used as the reference triterpenes. Extraction of lipid from air-dried materials was performed by either of the following four methods: (a), extracted with CHCl_3 -MeOH (2:1) at room temp. under stirring; (b) and (c), with CH_2Cl_2 and CHCl_3 in a Soxhlet extractor, respectively; and (d), with MeOH under reflux, and the extract was then treated with cold acetone to remove insoluble phospholipid; the acetone soluble portion was subjected to subsequent work. The unsaponifiable lipid obtained from the extract was separated, on preparative TLC, into two triterpene alcohol fractions: one contained the triterpenes with an axially oriented 3β -monohydroxyl group [referred to as triterpene (A)] ($R_f=0.53$) such as (1), and the other contained the usual triterpenes⁹⁾ with an equatorial 3β -monohydroxyl group [referred to as triterpene (B)] ($R_f=0.44$); in addition to sterol ($R_f=0.28$)⁸⁾ and several other fractions. The triterpene (A) fraction was acetylated and the resulted acetate fraction was subjected to GLC and GC-MS analyses.

3 Results and Discussion

Isolation and identification of the triterpenes from the triterpene (A) fractions of the seeds of *Trichosanthes japonica*, the pericarp of fruits and the aerial parts of *Sechium edule* was performed as described below.

Soxhlet extraction of the dried and ground seeds (500 g) of *T. japonica* afforded a lipid (106 g) from which was obtained an unsaponi-

fiable lipid (1.24 g). This was fractionated by preparative TLC giving triterpene (A) fraction (143 mg) which was then acetylated to yield the acetate (140 mg). GLC showed that the acetate was constituted of only one component with $\text{RRT}=1.43$ (mp. $117\sim118^\circ\text{C}$) which afforded the following spectroscopic data: MS: m/z 468 (M^+ , rel. int. 5%), 453 (3%), 408 (18%), 383 (12%), 298 (3%), 274 (100%), 259 (68%), 255 (5%), 231 (11%), 205 (12%). ^1H NMR: δ 0.81 (3 H, s, 18-H_3), 0.85 (3 H, s, 32-H_3), 0.91 (3 H, s, 19-H_3), 1.04 (6 H, s, 30-H_3 , 31-H_3), 2.01 (3 H, s, $3\beta\text{-OAc}$), 0.88 (3 H, d, $J=5.9$ Hz, 21-H_3), 1.59 (3 H, s, 27-H_3), 1.68 (3 H, s, 26-H_3), 4.70 (1 H, m, $W_{1/2}=5.9$ Hz, $3\alpha\text{-H}$), 5.51 (1 H, m, $W_{1/2}=10.0$ Hz, 6-H), 5.09 (1 H, t, $J=7.3$ Hz, 24-H). The assignment of the proton signals, done as above, was performed by the ^1H NMR study using a lanthanide shift reagent¹⁰⁾. The chromatographic and spectroscopic data were consistent with those of authentic cucurbita-5,24-dienol (1) acetate (lit.⁵⁾ mp $115\sim117^\circ\text{C}$), and, hence, the triterpene was identified as the acetate of (1).

Soxhlet extraction of the dried and ground pericarp (720 g) from the fruits (9610 g) of *S. edule* gave a lipid (24 g). The unsaponifiable lipid (919 mg) obtained from the lipid was subjected to preparative TLC yielding triterpene (A) fraction (7.0 mg) which was then acetylated to give an acetate fraction (7.1 mg). GLC of the acetate showed that it to be a mixture of the components with RRT 1.43 (3%) and 1.89 (87%) accompanied with several other components (10%). The triterpene with RRT 1.43 was identified as the acetate of (1) by the GLC and GC-MS data. Crystallization of the acetate fraction enabled the enrichment of the component with RRT 1.89 (96% pure by GLC, 3 mg) (mp $191\sim194^\circ\text{C}$) which provided the following spectroscopic data: MS: m/z 468 (M^+ , rel. int. 1%), 408 (81%), 393 (23

Table-1 Cucurbitaceae plants investigated, triterpene (A) contents of dried plant materials, and triterpene (A) compositions (%).

Cucurbitaceae	Plant material	Lipid extraction ^{a)}	Content of triterpene (A) mixture ^{b)}	Compositions (%)			
				(1)	(2)	(3)	Others
<i>Apodanthera undulata</i> Gray	Seeds	(a)	5.9	23			77
<i>Benincasa cerifera</i> Savi (wax gourd)	Seeds	(b)	—	2	16	2	80
<i>Citrullus battich</i> Forskål (watermelon)	Seeds	(b)	17.2	100			
	Aerial parts	(d)	11.5	11	35		44
<i>Cucumis melo</i> L. (melon)	Seeds	(b)	16.3	100			
<i>C. sativus</i> L. (cucumber)	Seeds	(b)	2.3	8			92
	Aerial parts	(d)	19.9	11	40		49
<i>Cucurbita digitata</i>	Seeds	(a)	59.4	100			
<i>C. foetidissima</i> HBK (buffalo gourd)	Seeds	(a)	13.5	23			77
<i>C. maxima</i> Duschesne (squash)	Seeds	(b)	—	41			59
<i>C. pepo</i> L. (pumpkin)	Seeds	(b)	—	100			
<i>Gynostemma pentaphyllum</i> Makino	Aerial parts	(d)	7.8	11			89
<i>Lagenaria leucantha</i> Rusby var. Gourda Makino (bottle gourd)	Seeds	(b)	41.3	100			
<i>L. leucantha</i> Rusby var. <i>depressa</i> Makino	Seeds	(b)	28.8	100			
	Pericarp	(b)	1.0	71			29
<i>L. leucantha</i> Rusby	Seeds	(c)	—	100			
<i>Luffa acutangula</i> Roxb.	Seeds	(c)	—	22	3		75
<i>L. cylindrica</i> Roem. (sponge gourd)	Seeds	(b)	10.0	100			
<i>Momordcia charantia</i> L. (balsam pear)	Seeds	(b)	9.8	100			
	Aerial parts	(c)	—	100			
<i>M. charantia</i> L. var. <i>Pavel</i> Crantz	Seeds	(b)	2.5	44			45
<i>M. cochinchinensis</i> Spreng	Seeds	(c)	—	1	70		29
<i>Sechium edule</i> Sw. (chayote)	Aerial parts	(d)	2.7	4	5	88	3
	Pericarp	(b)	1.0	2	87		10
<i>Trichosanthes anquina</i> L. (serpent cucumber)	Seeds	(c)	—	5	58		37
<i>T. dioica</i>	Roots	(c)	—	100			
<i>T. japonica</i> Regel	Seeds	(b)	28.7	100			
	Roots	(d)	0.4	100			

a) See experimental section.

b) Contents of triterpene (A) (mg) from dried plant material (seeds or mature plants) (100 g).

%), 274 (66%), 259 (59%), 205 (37%), 187 (30%), 173 (62%), 134 (92%), 121 (96%), 109 (100%). ¹H NMR⁽¹⁰⁾: δ 0.85 (3 H, s, 25-H₃), 0.95 (3 H, s, 29-H₃), 0.99 (3 H, s, 30-H₃), 1.00 (3 H, s, 26-H₃), 1.04 (3 H, s, 24-H₃), 1.07 (3 H, s, 23-H₃), 1.10 (3 H, s, 27-H₃), 1.16 (3 H, s, 28-H₃), 2.01 (3 H, s, 3 β -OAc), 4.69 (1 H, m, $W_{1/2}$ =4.7 Hz, 3 α -H), 5.56 (1 H, m, $w_{1/2}$ =7.4 Hz, 6-H). These data were almost identical with those of authentic glutinol (2) acetate (lit.⁽¹¹⁾ mp 192~194°C), and, hence, the triterpene with RRT 1.89 was identified as the acetate of (2).

Extraction with MeOH of the dried and ground aerial parts (1220 g) of *S. edule* gave a lipid (93 g) which on treatment with cold acetone gave an acetone soluble lipid (29 g). Saponification of the lipid yielded an unsaponifiable lipid (1.37 g) from which was obtained triterpene (A) fraction (34 mg) by preparative TLC. This was acetylated, and the resulted acetate fraction was shown by GLC to contain the components with RRT 1.43 (5%), 1.89 (4%), and 2.40 (88%), in addition to several other components (3%). Two triterpenes with RRT 1.43 and 1.89 were identi-

fied as the acetates of (1) and (2), respectively, by GLC and GC-MS. Crystallization of the acetate fraction enabled the enrichment of the component with RRT 2.40 (98% pure by GLC, 8 mg) (mp 217~218°C) which exhibited the following spectroscopic data: MS: m/z 468 (M^+ , rel. int. 1%), 453 (2%), 408 (22%), 393 (10%), 274 (93%), 259 (100%), 245 (13%), 236 (32%), 207 (26%), 134 (95%), 122 (98%). 1H NMR¹⁰: δ 0.78 (3 H, s, 28- H_3), 0.89 (3 H, s, 25- H_3), 0.92 (3 H, s, 26- H_3), 1.01 (3 H, s, 27- H_3), 1.04 (3 H, s, 24- H_3), 1.07 (3 H, s, 23- H_3), 2.02 (3 H, s, 3 β -OAc), 0.82 and 0.89 (each 3 H, d, and $J=6.3$ Hz, 29- H_3 , 30- H_3), 4.69 (1 H, m, $W_{1/2}=4.7$ Hz, 3 α -H), 5.54 (1 H, m, $W_{1/2}=7.4$ Hz, 6-H). These data were consistent with those of authentic simiarenol (3) acetate (lit.¹²) mp. 216~217°C). Hence, the triterpene with RRT 2.40 was identified as the acetate of (3).

Identification of the triterpenes (1), (2), and (3) in the plant materials, other than those described above, was performed by GLC and GC-MS. Table-1 showed the contents of triterpene (A) fractions of the dried plant materials which were determined based on the amounts of the triterpene (A) fractions recovered, and the compositions of the triterpene (A) fractions which were determined by GLC. Although the natural occurrence of the triterpene (1) has heretofore been known only in the seeds of *Lagenaria leucantha* var. *Gourda*, *Citrullus battich*, *Cucurbita pepo*, and *Trichosanthes kirilowii*⁵, this study demonstrated the widespread occurrence of the triterpene in the seeds and, furthermore, in the mature plant tissues of the cucurbitaceous plants including the above four seed materials. This study has further demonstrated the occurrence of two other triterpenes, (2)^{11,18} and (3)^{12,14,15}, which represented the rare classes of triterpenes, in some of the Cucurbitaceae investigated.

The triterpene (1) is considered to be biosynthesized directly from squalene-2, 3-oxide¹⁶, and which, on the other hand, is regarded as a key intermediate of the biosynthesis of cucurbitacins⁹ found in Cucurbitaceae^{1,2}. The co-occurrence of the triterpenes (2) and (3) with (1), all of which possess an axial 3 β -

hydroxyl group and a Δ^5 -bond, in some cucurbitaceous plant materials is of interest from the biogenetic viewpoint of triterpenoids. The constitutions of the triterpene (B) fractions⁹, which contain the triterpenes possessing a common equatorial 3 β -hydroxyl group, of Cucurbitaceae are under studying.

Acknowledgment

The authors are grateful to Drs. T. Takido and M. Aimi for the NMR and mass spectra.

(Received July. 10, 1986)

References

- 1) D. Lavie and E. Glotter, *Fortschr. Chem. Org. Naturst.*, **29**, 307 (1971).
- 2) M.C. Das and S.B. Mahato, *Phytochemistry*, **22**, 1071 (1983).
- 3) B. Shohat, S. Gitter, B. Lavie, and D. Lavie *Cancer Res.*, **25**, 1828 (1965).
- 4) S.M. Kupchan, R.M. Smith, Y. Aynechi, and M. Maruyama, *J. Org. Chem.*, **35**, 2891 (1970).
- 5) T. Itoh, T. Tamura, T.M. Jeong, T. Tamura, and T. Matsumoto, *Lipids*, **15**, 122 (1980).
- 6) G. Balliano, O. Caputo, F. Viola, L. Delprino, and L. Cattel, *Phytochemistry*, **22**, 909 (1983).
- 7) T. Itoh, H. Tani, K. Fukushima, T. Tamura, and T. Matsumoto, *J. Chromatogr.*, **234**, 65 (1982).
- 8) T. Akihisa, P. Ghosh, S. Thakur, F.U. Rosenstein, and T. Matsumoto, *J. Am. Oil Chem. Soc.*, **63**, 653 (1986).
- 9) T. Itoh, T. Shigemoto, N. Shimizu, T. Tamura, and T. Matsumoto, *Phytochemistry*, **21**, 2414 (1982).
- 10) T. Akihisa, N. Shimizu, R. Kawaguchi, T. Tamura, and T. Matsumoto, *J. Jpn. Oil Chem. Soc.*, **35**, 907 (1986).
- 11) F.G. Fischer and N. Seiler, *Liebigs Ann. Chem.*, **644**, 162 (1961).
- 12) K. Nishimoto, M. Ito, S. Natori, and T. Ohmoto, *Tetrahedron*, **24**, 735 (1968).
- 13) T. Ohmoto, M. Ikuse, and S. Natori, *Phytochemistry*, **9**, 2137 (1970).
- 14) H.R. Arthur, S.W. Tam, and V. Angsusingh, *Aust. J. Chem.*, **13**, 506 (1960); R.T. Aplin, H.R. Arthur, and W.H. Hui, *J. Chem. Soc. (C)* 1251 (1966).
- 15) L. Ogunkoya, O.O. Olubajo, and D.S. Sondha, *Phytochemistry*, **11**, 3093 (1972).

うり科植物に広範囲に分布する
ククルビタ-5,24-ジエノール

秋久俊博*・P. Ghosh**・S. Thakur**
F.U. Rosenstein***

田村利武*・松本太郎*

* 日本大学理学部 (〒101 千代田区神田駿河台 1-8)

** Burdwan 大学化学科 (インド Burdwan 市)

*** アリゾナ大学農学部栄養食糧科学科
(米国アリゾナ州ツーソン市)

うり科の 11 属の植物から得られた 28 種類にのぼる

種子及び成熟組織(茎・葉部, 果肉, 根)の抽出脂質のすべてに, Δ^5 -結合及び 3β -ヒドロキシル基をもつククルビタン系トリテルペンであるククルビタ-5,24-ジエノールの存在を明らかにした。さらに, いくつかのうり科植物試料には Δ^5 -結合をもつ五環性トリテルペンアルコールであるグルチノールとシミアレノールも検出した。ククルビタ-5,24-ジエノールは, うり科植物に存在の知られるククルビタシン類の生合成における重要な一中間体とみなされる。