Arch Pharm Res Vol 22, No 6, 629-632, 1999

Lupane-Glycoside of *Acanthopanax trifoliatus* forma *tristigmatis* Leaves

Chang-Soo Yook, Seung-Yeup Chang, Joung Hshiang Lai[1], Sung Kwon Ko, Jin-Hyun Jeong and Toshihiro Nohara[2]

College of Pharmacy, Kyung Hee University, Seoul, Korea, 130-701 and Wen Hua International Pharmacognostical institute, Fen-yen, Thai-Chung, ROC[1], [2]Department of Pharmacy, Kumamoto University, 860, Japan

(Received July 7, 1999)

This report contains the first characterization of acanthodiolglycoside which belongs to pentacyclic lupane triterpene glycoside

Key words: Lupane-glycoside, *Acanthopanax trifoliatus*, tristigmatis leaves

INTRODUCTION

Acanthopanax trifoliatus forma *tristigmatis* C. S. Yook et J. H. Lai belongs to Araliaceae which is a kind of vine-creeps-type shrubs. Its height is about 1-6 m. Its leaves are quite glossy due to transudation and its roots are shaped to three-forked style. It jąs been utilized as a folk-medicine for bruise, neuralgia, impotence, and gout in China, Taiwan, and the Philippines. Studies of *Acanthopanax trifoliatus* has been extensively reported; such as a publication about 3α,11α-23trihydroxylup-20(29)-en-28-oic acid by M. Lischewski, V. Phiet, A. Preiss, J. Schimid, and G. Adam in 1984 (Kutschabsky, *et al.*, 1985), a publication about 24-nor-11α-hydroxy-3-oxo-lup-20(29)-en-28-oic acid, and 24-nor-3α,11α-dihydroxy -lup-20(29)-enoic acid as new compounds by M. Lischewski, D. Pfeiffer, T. V. Sung, G. Adam in 1985 (Lischewski, *et al.*, 1985), a publication about 3α,11α-dihydroxy-23-oxo-lup-20(29)-en-28-oic acid, a free triterpene, by M. Lischewski, V. Phiet, V. Nguyen, and G. Adam in 1985 (Ty, *et al.*, 1985), a publication about the isolation

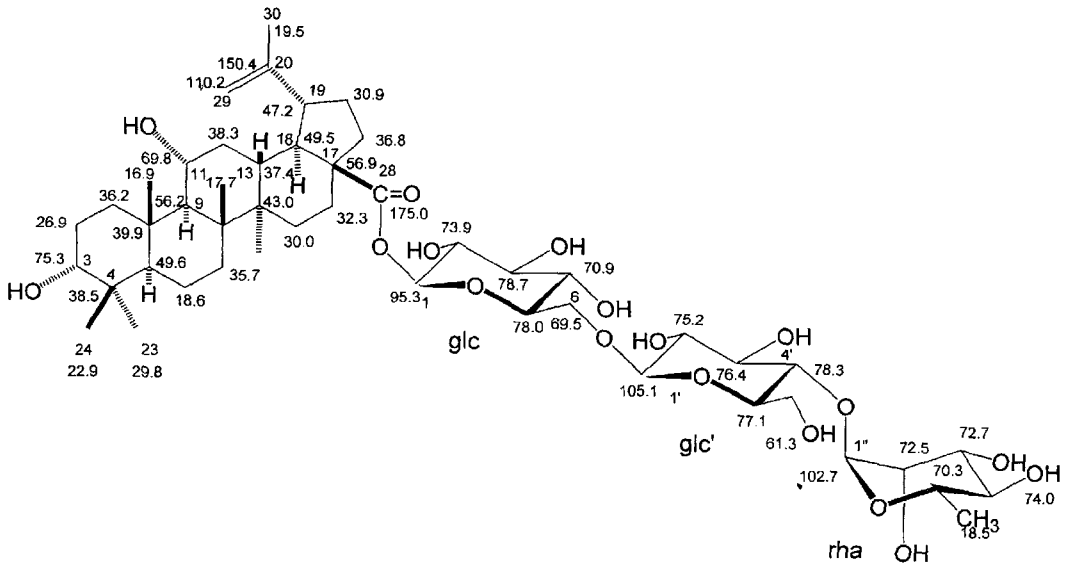

Fig. 1. Structrue of Acanbtrifoside A

Correspondence to: Professor Chang-Soo Yook, College of Pharmacy, Kyung Hee University, Seoul, Korea, 130-710
E-mail: Yookcs@nms.kyunghee.ac.kr

Table I. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectral data of compound **1** in pyridine-d_5 (δ values in ppm)

C	δ_C	δ_H *	Cross peaks ($_C$) in HMBC spectrum
1	36.2 CH_2	2.22 (*m*†)	26.9 (2), 49.6 (5), 75.3 (3)
		3.08 (*br d*, 12.8)	
2	26.9 CH_2	1.78 (*m*†), 2.15 (*m*†)	36.2 (1)
3	75.3 CH	3.61 (*br s*)	22.9 (24) 36.2 (1), 49.6 (5)
4	38.5 C		
5	49.6 CH	1.75 (*m*)	18.6 (6), 22.9 (24), 38.5 (4)
6	18.6 CH_2	1.38 (*m*†), 1.50 (*m*†)	49.6 (5), 35.7 (7)
7	35.7 CH_2	1.34 (*m*†), 1.47 (*m*†)	56.2 (9)
8	42.8 C		
9	56.2 CH	1.83 (*d*, 10.4)	16.9 (25), 35.7 (7), 39.9 (10), 42.8 (8), 69.8 (11)
10	39.9 C		
11	69.8 CH	4.27 (*m*†)	
12	38.3 CH_2	1.58 (*m*†), 2.36 (*m*)	37.4 (13), 69.8 (11)
13	37.4 CH	2.85 (*m*)	14.8 (27), 43.0 (14) 49.5 (18)
14	43.0 C		
15	30.0 CH_2	1.19 (*m*†), 1.94 (*m*)	43.0 (14)
16	32.3 CH_2	1.51 (*m*†)	43.0 (14), 49.5 (18), 56.9 (17)
		2.63 (*dt*, 12.8)	
17	56.9 C		
18	49.5 CH	1.70 (*m*†)	37.4 (13), 47.2 (19), 56.9 (17), 150.4 (20), 175.0 (28)
19	47.2 CH	3.37 (*m*)	
20	150.4 C		
21	30.9 CH_2	1.41 (*m*†), 2.14 (*m*†)	36.8 (22), 47.2 (19), 49.5 (18)
22	36.8 CH_2	1.47 (*m*†), 2.18 (*m*†)	30.9 (21), 56.9 (17), 49.5 (18)
23	29.8 CH_3	1.23 (*s*†)	22.9 (24), 38.5 (4), 75.3 (3)
24	22.9 CH_3	0.96 (*s*)	29.8 (23), 38.5 (4), 49.6 (5), 75.3 (3)
25	16.9 CH_3	1.26 (*s*)	36.2 (1), 39.9 (10), 49.6 (5), 56.2 (9)
26	17.7 CH_3	1.23 (*s*†)	35.7 (7), 43.0 (14), 56.2 (9)
27	14.8 CH_3	0.98 (*s*)	30.0 (15), 37.4 (13), 42.8 (8), 43.0 (14)
28	175.0 C		
29	110.2 CH_2	4.61 (*br s*)	19.5 (30), 47.2 (19)
		4.80 (*br s*)	
30	19.5 CH_3	1.65 (*s*)	47.2 (19), 110.2 (29), 150.4 (20)
C-28	O-inner gluc		
1	95.3 CH	6.30 (*d*, 7.9)	175.0 (28)
2	73.9 CH	4.07 (*m*†)	78.7 (g-3)
3	78.7 CH	4.19 (*m*†)	70.9 (g-4), 73.9 (g-2), 78.0 (g-5)
4	70.9 CH	4.29 (*m*†)	78.7 (g-3)
5	78.0 CH	4.09 (*m*†)	95.3 (g-1)
6	69.5 CH_2	4.27 (*m*†)	
		4.66 (α, 11.6)	105.1 (g-1')
glc'(16)glc			
1'	105.1 CH	4.93 (*d*, 7.9)	69.5 (g-6)
2'	75.2 CH	3.92 (*t*, 8.5)	76.4 (g-3'), 105.1 (g-1')
3'	76.4 CH	4.11 (*m*†)	75.2 (g-2'), 78.3 (g-4')
4'	78.3 CH	4.36 (*t*, 9.2)	75.2 (g-2'), 77.1 (g-5'), 102.7 (r-1)
5'	77.1 CH	3.64 (*dt*, 9.2)	78.3 (g-4')
6'	61.3 CH_2	4.08 (*m*†), 4.19 (*m*†)	
rha (14)glc'			
1	102.7 CH	5.80 (*br s*)	70.3 (r-5), 72.7 (r-3), 78.3 (g-4')
2	72.5 CH	4.64 (*br s*)	70.3 (r-5), 72.7 (r-3)
3	72.7 CH	4.51 (*dd*, 9.2, 3.1)	74.0 (r-4)
4	74.0 CH	4.33 (*m*)	18.5 (r-6), 70.3 (r-5), 72.5 (r-2)
5	70.3 CH	4.93 (*m*)	
6	18.5 CH_3	1.68 (*d*, 6.1)	70.3 (r-5), 74.0 (r-4)

glc, β-D-glucopyranosyl; rha, α-L-rhamnopyranosyl.
All assignments of ^{1}H and ^{13}C signals were conformed by ^{1}H-^{1}H COSY, HMQC and HMBC spectra.
* J values (in Hz) in parentheses.
† Overlapped signals.

and characterization of diterpene (pimaric acid), lupane triterpene, ursane triterpene, phytosterol from its leaves by Kim, Jung-Tae and Yook, Chang-Soo in 1988. (Ty, *et al.*, 1984; Yook and Kim, 1990)

This report contains the first characterization of acanthodiolglycoside which belongs to pentacyclic lupane

triterpene glycoside.

MATERIALS AND METHODS

Materials

Materials made a collection of *Acanthopanax trifoliatus* forma *tristigmatis* from the area of Taichung and Yangming Mountain in February, 1983. The leaves from them was dried in the shade. The instruments for elucidation of structure was used as follows; Brucker AM-500 (^{1}H and ^{13}C NMR), GC-MS/ MS-DS, TSQ-700 (EI-Mass), Nicolet 2R-435.

Extraction & Isolation

500 g of Plant materials was crashed to the powder and extracted with 2 L of methanol twice for 4 h. The combined solution was concentrated to obtain about 90 g of ex. This ex. from methanol was added to water and partitioned to ether layer and non-ether layer. These non-ether layer was concentrated and separated by silica gel (230-400 mesh) column and recrystallized three times with methanol to give 70 mg of white crystal.

Purification of compound 1

Compound **1** which appeared to one spot was chromatographed by silica gel, and recrystallized three times with methanol. This material was identified as a single compound by one-spot after two-dimensional TLC development.(*n*-BuOH : Acetic acid : H_2O=4 : 1 : 2). Particularly, this material was responsed positively in the Leiberman-Buchard reaction.

RESULTS AND DISCUSSION

Compound 1

Compound **1** which was responsed positively in the Leiberman-Buchard reaction was identified as triterpenoid. the IR spectra showed in peaks as follows; 3410 (OH), 2926 (C-H), 1732 (C=O), 1640 (aromatic C=O), 1065 (C-OH). Rhamnose as the terminal sugar moiety was elucidated from the fragmentations of m/z, 942 and 796 in EIMS spectrum. In the 1 H NMR spectrum, chemical shift of 6.31 ppm (1 H, d, J=8.1 Hz), 4.92 ppm (1 H, d, J=7.6 Hz), and 5.82 ppm (1 H, s) corresponded to each anomeric hydrogen of 28-)-glucose (inner and outer) and 28-*O*-gamma-rhamnose (terminal). The ^{13}C NMR spectra of compound **1** was compared with that of chiisanoside as reference which came from major component of Orgapii in Giri mountain. Furthermore, the spectra of 3,4-seco-lup-triterpenoid glycosyl ester was compared with assignment for spectrum of compound **1**. From the comparison with the ^{13}C NMR spectrum of acanthodiol which is an aglycone of compound **1**, the increment of chemical shift at C-28 suggested that sugar moiety should be connected at C-28. If sugar moiety is connected at C-3, its peak appears at 80-82 ppm. If not, its peak appears at 73-76 ppm. In our case, peak was shown at 75.2 ppm which indicated that sugar moiety should be connected at C-28 rather than C-3.

Hydrolysis of Compound 1

50 mg of compound **1** was dissolved in water, followed by addition of 2% H_2SO_4, and hydrolyzed for 4h. The resultant was diluted by water and extracted by chloroform. The organic layer was separated, concentrated, and recrystallized by ethanol three times to obtain needle-like crystal which is an aglycon. This material was responded positively in the Liebermann-Burchard reaction. From the investigation of ^{13}C NMR spectrum and mass spectrum, it was visualized to a kind of C_{30} triterpennoid compound and m/z 43 peak of mass spectrum was came from the detachement of isopropenyl group at E-ring. Melting point of compound **1** was 240-241°C. The mixed melting point-experiment with acanthodiol which was purified from three-leaves orgalpi showed the constancy of melting point. The TLC development with benzene and ethanol (95:5) gave the same result (Rf, 0.14). Other spectra data including to IR, ^{1}H-NMR, ^{13}C-NMR, and GC-Mass was the same with acanthodiol, respectively.

CONCLUSION

The charaterization of compound **1** which was obtained from methanol extract of three-leaves *Acanthopanax trifoliantus* forma *tristigmatis* C. S. Yook et J. H. Lai has been carried out. The melting point and other instrumental deta (IR, ^{1}H-NMR, GC-Mass, ^{13}C-NMR) were obtained and analyzed. Hydrolsis condition gave us the acanthodiolglycoside ($C_{48}H_{78}O_{18}$), 3α-11α-dihydroxylup-20(29)-en-28-oic acid. In particular, compound 1 was the first compound from the leaves of *Acanthopanax trifoliatus* forma *tristigmatis* C. S. Yook et J. H. Lai.

ACKNOWLEDGEMENTS

Financial support from the Research Management Department, Kyung Hee University (March, 1995) is gratefully acknowledged.

REFERENCES CITED

Bezdetko, G. N., German, A. V., Khasina, E. I., Shevchenko, V. P., Dardymov, I. V., Myasoedov, N. F., Barenboim, G. M. and Todorov, I. N., Study of the pharma-

cokinetics and mechanism of action of *Eleutherococcus senticosus* glycosides V. Metabolism and the kinetics of binding with blood serum components, *Khim. Farm. Zh.*, 16, 528-531 (1982).

Hahn, D. R., Kasai, R., Kim, J. H., Taniyasu, S. and Tanaka, O., *Chem. Pharm. Bull.*, 32, 1244. (1984).

Hikino, H., Takahashi, M., Otake, K. and Konno, C., Isolation and hypoglycemic activity of eleutherans A, B, C, D, E, F and G glycans of *Eleutherococcus senticosus* roots. J. Nat. Prod., 49, 293-297 (1986).

Kasai, R., Matsumoto, K., Taniyasu, S., Tanaka, O., Kim, J. H. and Hahn, D. R., *Chem. Pharm. Bull.* 34, 3284 (1986).

Kutschabsky, L., Pfeiffer, D., Lischewski, M., TY, Ph. D. and Adam G. *Croatica Chemica. Acta*, 58, 427 (1985).

Kutschabsky, L., Pfeiffer, D., Phiet, H. V., Preiss, A., Sung, T. V. and Adam G., Lupane triterpene from leaves of *Acanthopanax trifoliatus*, free acanthodiol, *Phytochemistry*, 24, 2355 (1985).

Nishiyama, N., Kamegaya, T., Iwai, A., Saito, H., Sanada, S., Ida, Y. and Shoji, J., Effect of *Eleutherococcus senticosus* and its components on sex- and learning-behaviors and tyrosine hydroxylase activities of adrenal gland and hypothalamic regions in chronic stressed mice, *Shoyakugaku Zasshi*, 39, 238-242 (1985).

Shao, C. J., Kasai, R., Xu, J. D. and Tanaka, O., Saponins from leaves of *Acanthopanax senticosus* Harms, Ciwujia: structures of ciwujianosides B, C_1, C_2, C_3, C_4, D_1, D_2 and E, *Chem. Pharm. Bull.*, 36, 601-608 (1988).

Shao, C. J., Kasai, R., Xu, J. D. and Tanaka, O., Saponins from leaves of *Acanthopanax senticosus* Harms, Ciwujia: II. structures of ciwujianosides A_1, A_2, A_3, A_4, and D_3, *Chem. Pharm. Bull.*, 37, 42-45 (1989).

Suu, W. J. and Sha, Z. I., Determination of syringin in *Acanthopanax senticosus* by HPLC, *Bull. Chin. Mat. Med.*, 11, 234-235 (1986).

Takasugi, N., Moriguchi, T., Fuwa, T. S., Sanada, S., Ida, Y., Shoji, J. and Saito, H., Effect of *Eleutherococcus senticosus* and its components on rectal temperature, body and grip tones, motor coordination and exploratory and spontaneous movements in acute stressed mice, *Shoyakugaku Zasshi*, 39, 232-237 (1985).

Ty, P. D., Lischewski, M., Phiet, H. V., Preiss, A., Nguyen, Ph, V. and Adam, G. *Phytochemistry*, 24, 867 (1985).

Ty, P. D., Lischewski, M., Phiet, H. V., Preiss, A., Sung, T. V., Schmidt, J. and Adam, G. *Phytochemistry*, 23, 2889 (1984).

Xiang, R. D. and Xu, R.S., Studies on chemical constituents of the root bark of *Acanthopanax gracilistylus* W. W. Smith., *Acta Bot. Sin.*, 25, 356-362 (1983).

Xu, Z. B., Tong, W. J. and Yang, G., Assay of active constituents in different parts of manyprickle acanthopanax (*Acanthopanax senticosus*), *Chin. Trad. Herb Drugs*, 15, 224-226 (1984).

Xu, Z. B. and Wang, M. Y., Content variation of some chemical constituents of Ci Wu Jia (*Acanthopanax senticosus*) during storage, *Chin. Trad. Herb Drugs*, 15, 15-17 (1984).

Yook, C. S. and Kim, C. T., Studues on the constituents of *Acanthopanax trifoliatus*, structure of taraxasterylacetate diterpene, Bull. K. H. *Pharma. Sci.*,18, 33-42 (1990).