Pharmaceutical Sciences Asia

Pharm Sci Asia 2020; 47 (4), 310-318
DOI:10.29090/psa.2020.04.020.0058

Review Article

Bryonolic acid: A review on its phytochemistry and biological activities

Satsawat Visansirikul[1*],
Pornpatsorn Lertphadungkit[1,2]

[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand
[2] Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand

***Corresponding author:**
Satsawat Visansirikul
satsawat.vis@mahidol.ac.th

KEYWORDS:
Bryonolic acid, Anti-allergy, Anticancer, Anti-inflammatory, Antioxidant, Neuroprotection

https://www.pharmacy.mahidol.ac.th/journal/
© Faculty of Pharmacy, Mahidol University (Thailand) 2020

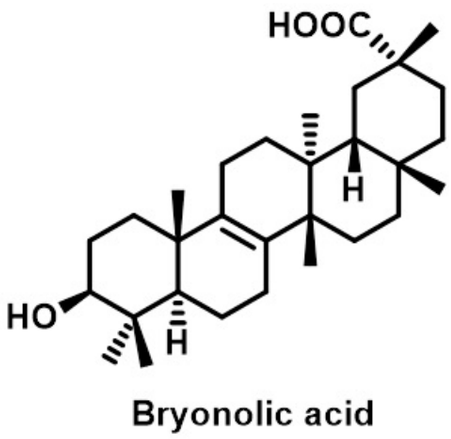
ABSTRACT

Bryonolic acid, pentacyclic triterpenoid, is found in Cucurbitaceae, Tetramelaceae, Meliaceae and Anisophylleaceae plant families. There were several previous studies reported some biological properties of bryonolic acid, such as anti-allergic effect in three types of allergy in mice. Moreover, it shown to inhibit growth of several cancer cell lines including melanoma, choriocarcinoma, hepatoma, epithelial carcinoma, fetal lung fibroblast, lymphosarcoma, lung cancer and breast cancer with acyl-coA: cholesterol acyl transferase (ACAT) inhibitory activity. Bryonolic acid was also proven to provide anti-inflammatory and anti-oxidation properties via activation of heme oxygenase 1 (HO-1) and reduction of nitric oxide level. Furthermore, bryonolic acid inhibited NMDA-induced exitotoxicity by decreasing intracellular Ca^{2+} concentration. Based on these properties, bryonolic acid could become an interesting compound for new drug development.

1. INTRODUCTION

Natural products are one of the most important sources of biological active molecules which can be used as lead compounds for new drugs discovery. These compounds have been isolating, identifying and characterizing continuously for many years. Triterpenoids is a class of diverse natural organic compounds from plants with various pharmacological activities which derived from squalene or six isoprene units.

Bryonolic acid, 3β-hydroxy-D:C-friedoolean-8en-29-oic acid[1] (Figure 1) belongs to pentacyclic triterpenoids. Unlike other pentacyclic triterpenoids, such as betulinic acid[2] and ursolic acid[3], bryonolic acid and its derivatives have not yet been widely studied on their pharmacological activities due to non-commercial availability. Therefore, bryonolic acid is an interesting compound which can be used as lead compound for structural modification in structure-activity relationship studies to develop new bioactive substances. Herein, we present a comprehensive review of bryonolic acid and its biological activities.



Bryonolic acid

Figure 1. Chemical structures of bryonolic acid

2. PHYTOCHEMISTRY OF BRYONOLIC ACID

2.1. Discovery and structure elucidation of bryonolic acid

In 1959, Biglino reported the first isolation of bryonolic acid from the roots of *Brynonia dioica* Jacq.. Its initial structure was known to comprise a C-19 carboxylic group and unsaturation at C-12/C-13[1]. After that, the chemical structure was revised to be an unsaturation at C-8/C-9 B-C ring fusion and a carboxyl group at C20[4] (Figure 1). Based on ^{1}H and ^{13}C NMR[4,5], the structure of bryonolic acid is known as 3β-hydroxy-D:C-friedoolean-8en-29-oic acid. The conformation of bryonolic acid was first reported by Kamisako et. al. in 1984 by performing the x-ray crystallography study of bryonolic acid derivative, D:C-friedoolean-8en-3β,29-diol diacetate (Figure 2, Compound 1)[6]. The conformation of ring A/B, C/D and D/E were found as trans, trans and cis respectively. The conformation of ring A to E are chair, half-chair, half-chair, twist-boat and boat respectively. However, the different substituent at the 20α position of bryonolic acid derivative might affect the conformation of the D-E ring moieties. Therefore, the conformation study of unmodified substituent at 20α position was provided subsequently by Nakai et. al. in 1987[7]. The methyl ester derivative of bryonolic acid (Figure 2, Compound 2) was subjected to x-ray crystallography study to yield the conformation of bryonolic acid. Rings A, B and C of bryonolic acid are found to be in chair, half-chair and half-chair conformation respectively. While both D and E rings are in chair form.

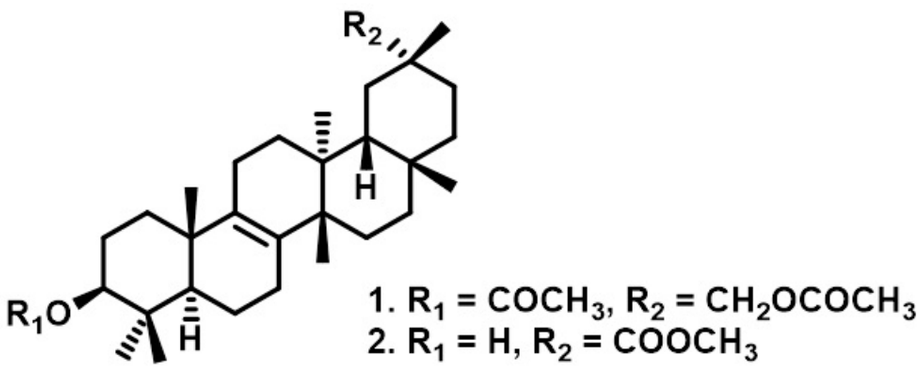


Figure 2. Chemical structures of D:C-friedoolean-8en-3β,29-diol diacetate (1) and methyl ester derivative of bryonolic acid (2)

2.2. Sources of bryonolic acid

Bryonolic acid is found in various Cucurbitaceae plants, such as *Benincasa cerifera* Savi (roots and radicle)[8], *Bryonia alba* (roots)[9], *Bryonia aspera* (roots)[10], *Brynonia dioica* (roots) [1], *Citrulluc lanatus* Matsum. et Nakai cv Zuisho (roots and radicle)[8], *Cucumis melo* L. cv Hasan bey (radicle)[8], *Cucumis melo* L. cv Kurkarch (radicle)[8], *Cucumis melo* L. cv New melon (roots and radicle)[8], *Cucumis melo* L. var. *conomon* Makino (roots and radicle)[8], *Cucumis sativus* L. cv Aonagayotsuba (radicle)[8], *Cucumis sativus* L. cv Asakaze (roots and radicle)[8], *Cucumis sativus* L. cv Tsubasa (radicle)[8], *Cucurbita moschata* Duch. cv Hayato (radicle)[8], *Cucurbita pepo* L. (seeds)[11], *Cucurbita pepo* L. cv Kintoga (radicle)[8], *Cucurbita pepo* L. var. ovifera Alef. (radicle)[8], *Lagenaria siceraria* Standl. (roots and radicle)[8], *Lagenaria siceraria* Standl. var *hispida* Hara (radicle)[8], *Lagenaria siceraria* Standl var *microcarpa* Hara (radicle)[8], *Luffa cylindrica* L. (roots)[12], *Luffa cylindrica* Roem. (roots and radicle)[8], *Momordica charantia* (roots)[13], *Trichosanthes bracteate* (roots)[14], *Trichosanthes cucumerina* (roots)[15], *Trichosanthes kirilowii* Maxim. (radicle)[8], *Trichosanthes kirilowii* Maxim. var. *japonica* Kitam (roots and radicle)[8], *Trichosanthes multiloba* (roots)[14], as well as in the Tetramelaceae[16] and Meliaceae[16] families, such as dried fruit of *Sandoricum indicum*[17]. In 2009, Khallaouki et. al. isolated bryonolic acid from the root barks of *Anisophyllea dichostyla* R. Br. which belonged to Anisophylleaceae family[18].

2.3. Biosynthesis of bryonolic acid

Bryonolic acid was purposed to be biosynthesized by the mevalonic acid pathway through squalene (Figure 3). In 1981, Cattel et. al. isolated isomultiflorenol from the seedlings of *B. dioica* which hypothesized to be a precursor of bryonolic acid[19]. However, this hypothesis has not been proven by experiment by that time. Cho et. al. reported the biosynthesis of bryonolic acid in cultured cell of watermelon in 1993[20]. They applied tracer experiments by using [2-^{14}C]acetate and R-[2-^{14}C]mevalonate as precursors of bryonolic acid in cell cultures. Lauryl dimethylamine N-oxide (LDAO), known as 2,3-oxidosqualene cyclase inhibitor and Tolnaftate, used as squalene expoxidase inhibitor were used to confirm the effect of these enzymes on biosynthesis pathway of bryonolic acid. Hence, Squalene is converted to 2,3-oxidosqualene by squalene epoxidase, followed by the cyclization of 2,3-oxidosqualene by 2,3-oxidosqualene cyclase which identified later as isomultiflorenol synthase by Hayashi et. al.[21] to yield dammarenyl cation. After that, sequential D-ring enlargement provide baccharenyl cation, followed by E-ring formation to acquire lupanyl cation. Then, a series of 1,2-hydride shifts and formation of unsaturated carbon through deprivation of C-9 hydrogen yield isomultiflorenol. Furthermore, recent study by Takase et. al. indicated that genes of isomultiflorenol synthase are more extensively expressed in the roots than the other parts of *Momordica charantia* which explained why bryonolic acid is highly synthesized in the roots[13]. Isomultiflorenol is then converted to bryonolol, bryonolal and bryonolic acid respectively by enzymatic oxidation reactions[20]. However, this oxidizing enzyme has not yet been identified.

3. BIOLOGICAL PROPERTIES OF BRYONOLIC ACID

3.1. Anti-allergy property of bryonolic acid

Tanaka et. al. isolated bryonolic acid from the cultured cells of *Luffa cylindrica* L. and compared anti-allergic effect with glycyrrhetinic acid, aglycone of glycyrrhizin[12] (figure 4). Due to the similarity in chemical structure between bryonolic acid and glycyrrhetinic acid which reported previously to exhibit anti-allergic activities, bryonolic acid might also have anti-allergic property. Hence, bryonolic acid was administered to rats by intraperitoneal route at doses of 300 and 600mg/kg and shown to inhibit homologous passive cutaneous anaphylaxis 23.3 and 80.6 % respectively which was more effective than glycyrrhetinic acid at 600 mg/kg. Moreover, bryonolic acid was also found to inhibit delayed hypersensitivity in mice significantly which was inactive for glycyrrhetinic acid. Because of the lacking 11-oxo functional group in bryonolic acid, it shown only little toxicity and no visible side effects on rats without inhibiting the activity of the hepatic enzyme, 5α- and 5β-reductase which strongly inhibited by glycyrrhetinic acid.

Tabata et. al. studied anti-allergic properties of bryonolic acid and its synthetic derivatives on three types of allergies (type I, III and IV) which artificially induced in male ddY mice[22] (figure 5).

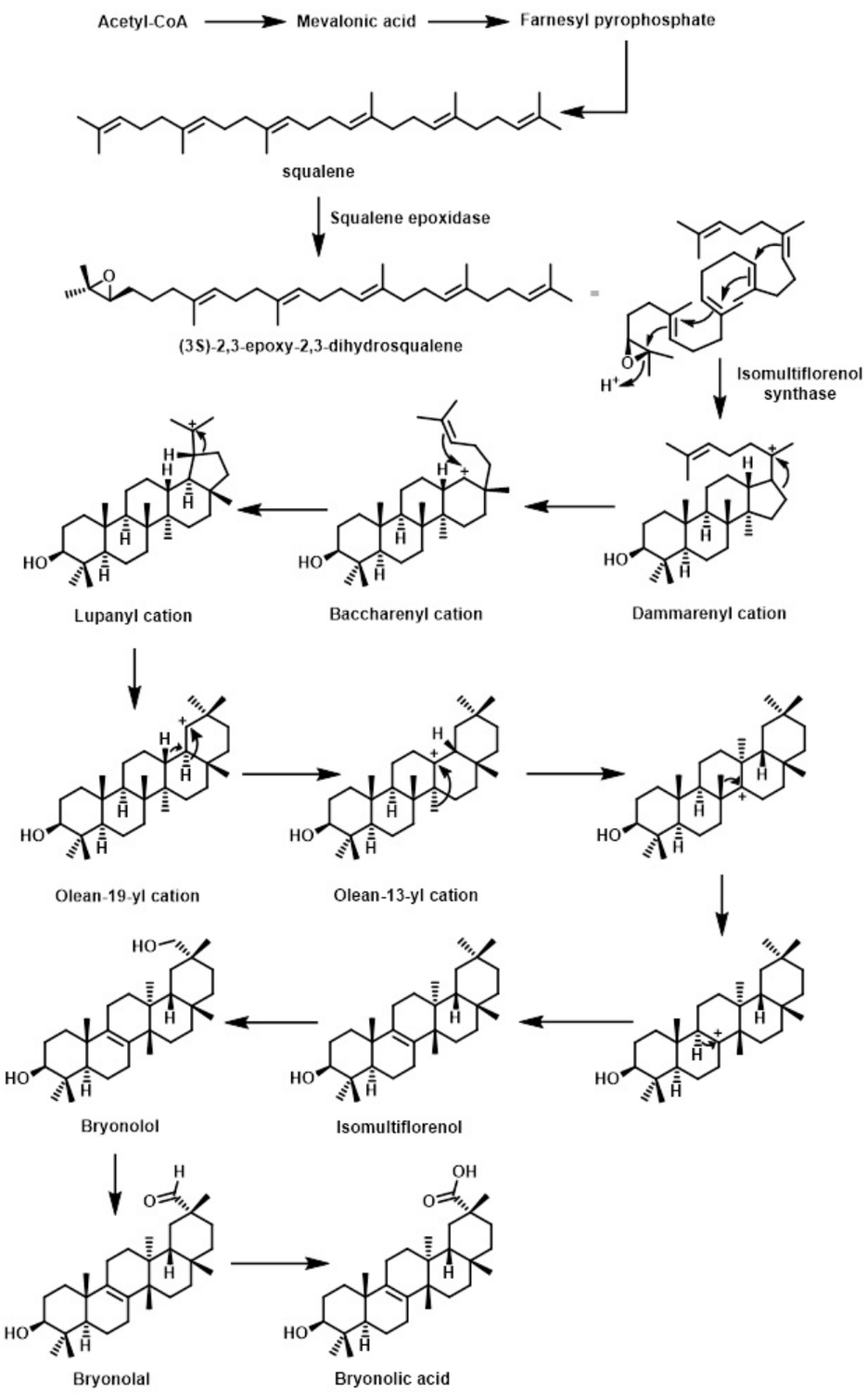


Figure 3. Biosynthesis pathway of bryonolic acid

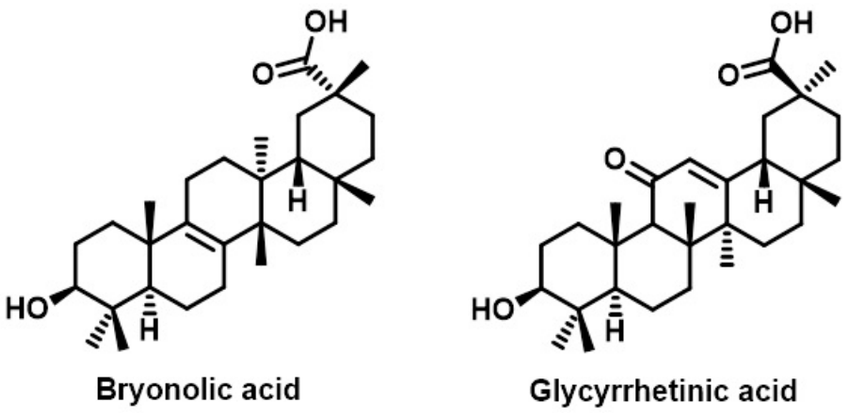


Figure 4. Chemical structures of bryonolic acid and glycyrrhetinic acid

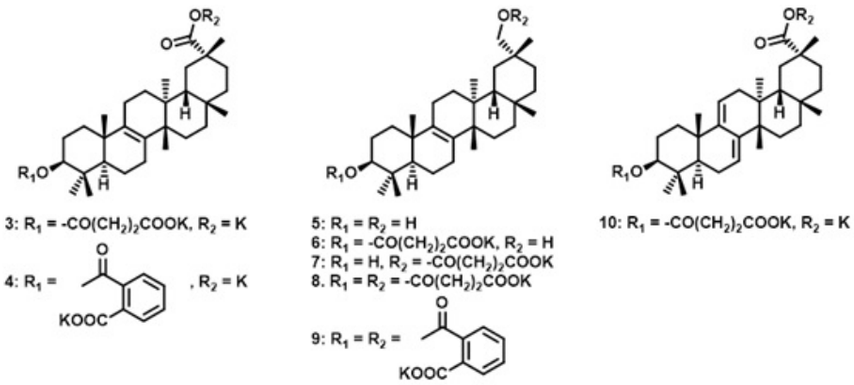


Figure 5. Chemical structure of synthetic bryonolic acid derivatives

Type I allergy was a study of their capability of inhibiting passive cutaneous anaphylaxis in mice by both intraperitoneal and oral administration. For intraperitoneal administration study, bryonolic acid and compound 7 had higher ID_{50} than reference drug, tranilast (ID_{50} = 376, >400 and 152 mg/kg respectively), while the rest of synthetic compounds were more reactive (ID_{50} was from 34.2 – 92.4 mg/kg). Three highly reactive compounds, 3, 4 and 9 (ID_{50} = 55.3, 34.2 and 41.4 mg/kg respectively), were subjected to oral administration study. Based on the results, compounds 3, bryonolic acid-3-succinate dipotassium, was the most active compound (ID_{50} = 63.7 mg/kg). Therefore, compound 3 was chosen to be a test compound for type III and IV allergy test.

Type III allergy assay was done by sheep-erythrocytes-induced Arthus reaction test in mice. As a result, the percent inhibition of orally administered compound 3 were 35.3 and 62.4 % at 300 and 600 mg/kg respectively, whereas the percent inhibition of reference drug, prednisolone succinate was 37.4 % at 30mg/kg.

Type IV allergy test was performed by picryl-chloride induced contact dermatitis assay in mice. The percent inhibition of orally administered compound 3 at doses between 75 to 300 mg/kg were 34.0 - 53.0 % respectively while prednisolone succinate at 30 mg/kg inhibits by 41.0%.

3.2. Anticancer property of bryonolic acid

Tekeda et. al. isolated bryonolic acid from the roots of *Trichosantes kirilowii* MAX. var. *japonica* KITAM. and assayed the effect on the growth of B16 melanoma cells *in vitro* by the MTT method[23]. Bryonolic acid was found to inhibit the cell growth significantly at 5 µg/mL.

After that, Kondo et. al. studied cytotoxicity of bryonolic acid against various cells, such as B16 cells (mouse melanoma), BeWo cells (human choriocarcinoma), dRLh-84 cells (rat hepatoma),

HeLa cells (human epithelial carcinoma), P388-D1 cells (mouse lymphosarcoma), PLC/PRF/5 cells (human hepatoma), IMR-90 cells (human fetal lung fibloblast), SF-TY cells (human normal skin fibroblast) and human hepatocytes[24]. IC_{50} of bryonolic acid for the tumor cells and fibroblasts were in the range of 10 - 50 µg/mL, while those for human fetal lung fibroblast and hepatocytes were more than 80 µg/mL (Table 1). Moreover, to investigate more about the cell death mechanism, DNA fragmentation analysis was performed. Based on the appearance of DNA ladder of bryonolic acid treated HL-60RG cells, the cell death was triggered by bryonolic acid seemed to be apoptosis.

Akihisa et. al. reported the screening of cytotoxic activity of various multiflorane-type triterpenoids[25]. Eleven isolated compounds and thirty-eight derivatives, including bryonolic acid and bryonolic acid acetate were tested on the inhibition of Epstein–Barr virus early antigen (EBV-EA) activation which induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) as a tumor promoter in Raji cells. Both bryonolic acid and bryonolic acid acetate shown an inhibitory effect on EBV activation at 100 mol ratio and exhibited significant activity at 1000 mol ratio (87.7% and 85.9% respectively). Also, their structure-activity relationship indicated that C-3 oxo group or acetylated or benzoylated at C-3 hydroxyl group were prone to reduce cytotoxic activity.

Kongtun et. el. reported cytotoxicity of bryonolic acid against several cancer cells, including two lung cancer cell lines (A549 and SK-LU1), four human breast cancer cell lines (MDA-MB435, SKBR3, MCF-7 and T47D) and one colon cancer cell (caco-2)[15]. Bryonolic acid had IC_{50} value against various cancer cell lines were from 90.5 to above 500 µg/mL (Table 1).

Table 1. IC_{50} of bryonolic acid against the tumor cells and human normal cells

Cells	IC_{50} (µg/mL)	Reference
B16	15	Kondo, 1995
PLC/PRF/5	20	
BeWo	24	
P388-D1	29	
dRLh-84	30	
HeLa	51	
SF-TY	51	
IMR-90	83	
Hepatocyte	92	Kongton, 2009
A549	99.7	
SK-LU1MDA-	>500	
MB435	90.5	
SKBR3,	131.9	
MCF-7	121.3	
T47D	124.1	
Caco-2	>500	

Khallouki et. al. studied the effect of bryonolic acid on the inhibition of cancer cell clonogenicity and invasiveness through the inhibition of cholesterol fatty acid ester formation[26]. Bryonolic acid inhibited acyl-coA: cholesterol acyl transferase (ACAT) activity in rat liver microsomes concentration dependently, blocking the biosynthesis of the cholesterol fatty acid ester tumor promoter with IC_{50} of 12.6 µM. It was also evaluated for ACAT inhibitory activity of various cancer cell

lines which IC_{50} values were reported as 22.5 µM for MCF-7, 29.5 µM for MB-231, 17.5 µM for U-87 and 19.4 µM for 3T3-EA. Furthermore, bryonolic acid at 25 µM shown more than 50% inhibition of cancer cell colony formation in four different cell lines mentioned earlier.

3.3 Anti-inflammatory and anti-oxidation properties of bryonolic acid

Barker et. al. investigated anti-inflammatory activity of bryonolic acid and found that bryonolic acid was a robust inducer of antioxidant protein heme oxygenase 1 (HO-1)[16]. After 24 hours of treatments of bryonolic acid in RAW 264.7 macrophage cells, HO-1 expression was induced by 3.3 fold and 14 fold compared to LPS control and 13 fold and 55 fold compared to untreated cells in the presence of 50µM and 100µM pf bryonolic acid respectively.

Gatbonton-Schwager et. al. studied the mechanism of the anti-inflammatory effect of bryonolic acid[27]. Bryonolic acid exhibited potent anti-inflammatory activity by reducing NO levels and activating HO-1 protein. Bryonolic acid reduces the inflammatory mediator NO by suppressing of inducible nitric oxide synthease (iNOS) gene expression in LPS-activated Raw 264.7 macrophage cells in a dose-dependent and time-dependent manner. (IC_{50} = 53.3 µM). Antioxidant protein HO-1 is found to be induced by bryonolic acid via Nrf2/Keap1 pathway activation.

3.4 Neuroprotective property of bryonolic acid

Que et. al. investigated neuroprotective effect of bryonolic acid in an NMDA-induced rat adrenal pheochromocytoma cell line (PC12) and its mechanism[28]. Bryonolic acid at 100 and 1000 µM were found to inhibit NMDA-induced exitotoxicity significantly without inhibitory effect on basal growth of PC12. Moreover, bryonolic acid decreased the intracellular Ca^{2+} concentration in NMDA-induced PC12cells. In addition, bryonolic acid was found to upregulate protein and mRNA expression of Bcl-2, p-CREB and p-CaMKII and downregulate protein and mRNA expression of Bax. Based on these results, bryonolic acid protected PC12 cells against NMDA-induced apoptosis by inhibiting Ca^{2+} influx and regulating gene expression in Ca^{2+}-CaMKII-CERB signal pathway.

4. CONCLUSIONS AND OUTLOOK

Bryonolic acid, a pentacyclic triterpenoid, can be found in various plants in Cucurbitaceae, Tetramelaceae, Meliaceae and Anisophylleaceae families. The previous studies shown several biological effects of bryonolic, such as anti-allergic activity, anticancer activity, anti-inflammatory activity, anti-oxidation activity and neuroprotective activity.

Based on bryonolic acid structure, there are some functional groups that can be derivatized, including C-3 hydroxyl and C-20 carboxyl groups. There are several routes to modify bryonolic acid structure. For examples, both functional groups are available for either esterification or amide formation. Moreover, carboxyl group can be subjected to reduction reaction to yield aldehyde or alcohol derivatives. While hydroxyl group can be oxidized to ketone. Also, bryonolic acid glycosides, obtained by glycosylation reaction, should be other interesting derivatives since attached sugar compounds will provide hydrophilic groups and can improve pharmacokinetic properties of bryonolic acid. Based on these results, bryonolic acid with its unique properties may be considered as a promising natural lead compound for derivatization in order to obtain new semi-synthetic compounds which can be used as promising drug candidates in the future.

5. ACKNOWLEDGEMENTS

This work is supported by Faculty of Pharmacy, Mahidol University.

Conflict of interest (If any)

None to declared

Funding

New staff research fund from Faculty of Pharmacy, Mahidol University.

Ethical approval

None to declare

Article info:
Received April 18, 2020
Received in revised form May 12, 2020
Accepted May 19, 2020

REFERENCES

1. Biglino G. Constituents of the root of *Bryonia dioica*. Ann Chim. 1959;49:782-92.
2. Perumal Y, Sriram D. Betulinic acid and its derivatives: A review on their biological properties. Curr Med Chem. 2005;12:657-66.
3. López-Hortas L, Pérez-Larrán P, González-Muñoz MJ, Falqué E, Domínguez H. Recent developments on the extraction and application of ursolic acid. A review. Food Res. 2018;103: 130-49.
4. Biglino G, Cattel L, Caputo O. Structure of bryonolic acid. Ric Sci. 1969;39(3):207-9.
5. Kamisako W, Suwa K, Morimoto K, Isoi K. Application of biosynthetic 13C-enrichment using [1-13C]-, [2-13C]- and [1,2-13C2]-acetate as precursor for 13C NMR spectral assignment of higher plant metabolites. The assignments of some bryonolic acid derivatives. Org Magn Reson. 1984;22(2):93-100.
6. Kamisako W, Isoi K, Nakai H, Shiro M. Structure of a bryonolic acid derivative, D:C-friedoolean-8-ene-3β,29-diol diacetate, C34H54O4. Acta Crystallogr C. 1984;40(6): 1013-5.
7. Nakai H, Shiro M, Kamisako W, Honda C, Isoi K. Structure of a bryonolic acid derivative, 3β-hydroxy-D:C-friedoolean-8-en-29-oic acid methyl ester. Acta Crystallogr C. 1987;43: 1779-1782.
8. Cho HJ, Tanaka S, Fukui H, Tabata M. Formation of bryonolic acid in cucurbitaceous plants and their cell cultures. Phytochemistry. 1992;31(11): 3893-6.
9. Saltykova IA, Matyukhina LG, Shavva AG. Bryonolic acid in the roots of *Bryonia alba*. Chem Nat Compd. 1968;4(5):275.
10. Sahranavard S, Naghibi F, Siems K, Jenett-Siems K. New cucurbitane-type triterpenoids from *Bryonia aspera*. Planta medica. 2010;76 (10):1014-7.
11. Appendino G, Jakupovic J, Belloro E, Marchesini A. Triterpenoid p-aminobenzoates from the seeds of zucchini. Fitoterapia. 2000; 71(3):258-63.
12. Tanaka S, Uno C, Akimoto M, Tabata M, Honda C, Kamisako W. Anti-allergic effect of bryonolic acid from *Luffa cylindrica* cell suspension cultures. Planta Med. 1991;57(6): 527-30.
13. Takase S, Kera K, Hirao Y, Hosouchi T, Kotake Y, Nagashima Y, et al. Identification of triterpene biosynthetic genes from *Momordica charantia* using RNA-seq analysis. Biosci Biotech Bioch. 2019;83(2):251-61.
14. Kitajima J, Tanaka Y. Studies on the constituents of Trichosanthes root. IV. Constituents of roots of *Trichosanthes multiloba* Miq., Trichosanthes miyagii Hay. and Chinese crude drug karo-kon. *Yakugaku zasshi* : J Pharm Soc Japan. 1989;109(9):677-9.
15. Kongtun S, Jiratchariyakul W, Kummalue T, Tan-ariya P, Kunnachak S, Frahm AW. Cytotoxic properties of root extract and fruit juice of *Trichosanthes cucumerina*. Planta medica. 2009;75(8):839-42.
16. Barker EC, Gatbonton-Schwager TN, Han Y, Clay JE, Letterio JJ, Tochtrop GP. Bryonolic acid: a large-scale isolation and evaluation of heme oxygenase 1 expression in activated macrophages. J Nat Prod. 2010;73(6):1064-8.
17. Sim KY, Lee HT. Triterpenoid and other constituents from *Sandoricum indicum*. Phytochemistry. 1972;11(11):3341-3.
18. Khallouki F, Hull WE, Owen RW. Characterization of a rare triterpenoid and minor phenolic compounds in the root bark of *Anisophyllea dichostyla* R. Br. Food Chem Toxicol. 2009; 47(8):2007-12.
19. Cattel L, Balliano G, Caputo O, Viola F. Biosynthesis of cucurbitacins in *Bryonia dioica* seedlings. Planta Med. 1981;41(4):328-36.
20. Cho HJ, Ito M, Tanaka S, Kamisako W, Tabata M. Biosynthesis of bryonolic acid in cultured cells of watermelon. Phytochemistry. 1993;33(6): 1407-13.
21. Hayashi H, Huang P, Inoue K, Hiraoka N, Ikeshiro Y, Yazaki K, et al. Molecular cloning and characterization of isomultiflorenol synthase, a new triterpene synthase from Luffa cylindrica, involved in biosynthesis of bryonolic acid. Eur J Biochem. 2001;268(23): 6311-7.
22. Tabata M, Tanaka S, Cho HJ, Uno C, Shimakura J, Ito M, et al. Production of an anti-allergic triterpene bryonolic acid, by plant cell cultures. J Nat Prod. 1993;56(2):165-74.
23. Takeda T, Kondo T, Mizukami H, Ogihara Y. Bryonolic acid production in hairy roots of

Trichosanthes kirilowii Max. var Japonica Kitam. transformed with *Agrobacterium rhizogenes* and its cytotoxic activity. Chem Pharm Bull (Tokyo). 1994;42(3):730-2.
24. Kondo T, Inoue M, Mizukami H, Ogihara Y. Cytotoxic activity of bryonolic acid isolated from transformed hairy roots of *Trichosanthes kirilowii* var. japonica. Biol Pharm Bull. 1995; 18(5):726-9.
25. Akihisa T, Tokuda H, Ichiishi E, Mukainaka T, Toriumi M, Ukiya M, et al. Anti-tumor promoting effects of multiflorane-type triterpenoids and cytotoxic activity of karounidiol against human cancer cell lines. Cancer Lett. 2001;173(1):9-14.
26. Khallouki F, Owen RW, Silvente-Poirot S, Poirot M. Bryonolic acid blocks cancer cell clonogenicity and invasiveness through the Inhibition of fatty acid: cholesteryl ester formation. Biomedicines. 2018;6(1):21.
27. Gatbonton-Schwager TN, Letterio JJ, Tochtrop GP. Bryonolic acid transcriptional control of anti-inflammatory and antioxidant genes in macrophages *in vitro* and *in vivo*. *J Nat* Prod. 2012;75(4):591-8.
28. Que J, Ye M, Zhang Y, Xu W, Li H, Xu W, et al. Bryonolic acid, a triterpenoid, protect against N-methyl-d-aspartate-induced neurotoxicity in PC12 cells. Molecules. 2016;21(4):418.