Asian Journal of Chemistry Vol. 21, No. 5 (2009), 3892-3896

Linolein-2-Stearin Phosphate and Linolenic Acid β-D-Glucoside: The Newer Isolates of *Alpinia galanga* Rhizomes

SHIVKANYA B. JAJU*, N.H. INDURWADE, D.M. SAKARKAR, M. ALI†, N.K. FULORIA‡ and N.J. DURAGKAR§
Sudhakarrao Naik Institute of Pharmacy, Pusad-445 204, India
E-mail: shivani_jaju@rediffmail.com

Alpinia galanga willd rhizome on chemical analysis and sequential chromatography explored two newer compounds, Linolein-2-stearin phosphate and linolenic acid β-D-glucoside. Structures of newer compounds were elucidated by various spectral techniques (UV, IR, ^{1}H NMR, ^{13}C NMR and MS).

Key Words: *Alpinia galanga* Rhizomes, Linolein-2-Stearin Phosphate, Linolenic Acid β-D-Glucoside.

INTRODUCTION

Perennial herb *Alpinia galanga* belonging to Zingiberaceae family, mainly exists in the warmer parts of Asia, known with the common name greater galangal[1-4]. The genus Alpinia is well known to contain various essential oils such as cineole, methyl cinnamate, myrecene, methyl eugneol. and various flavones like galangin, alpinin, kampferide and 3-dioxy-4-methoxy flavone[5-7]. *Alpinia galanga* is known to possess antimicrobial activity[8], antioxidantactivity[9], antifungal activity[10], anticancer activity[11] and gastroprotective activity[12]. The present investigation reports linolein-2-stearin phosphate and linolenic acid β-D-glucoside isolated from rhizomes of *Alpinia galanga*. The method and isolated compounds in the present investigation is reported for first time.

EXPERIMENTAL

The dried rhizomes of *Alpinia galanga* (Zingiberaceae), collected in Pusad province of India were identified by Prof. AIka Chaturvedi Deparment of Botany, Nagpur, India. A voucher specimen is deposited in the Natural Medicine Research Center of this Institute.

Column chromatography and thin layer chromatography[13-15] were carried out using silica gel 60-120 mesh (Hi media) and silica gel G (Hi Media), respectively. IR spectra of the newly isolated compounds were recorded in a Jasco IR spectrophotometer using KBr pellet. The FT-NMR spectra (Bruker) were recorded using $CDCl_3$ as solvent and TMS as internal standard.

†Department of Pharmacy, Jamia Hamdard, New Delhi-110 062, India.
‡Ram-eesh Institute of Technical and Vocational Eduction, Greater Noida-201 306, India.
§S.P. College of Pharmacy, Nagpur-441 110, India.

Methanolic extraction and isolation of compounds using column chromatography: Dried, ground rhizome of *Alpinia galanga* (3000 g) was defatted with petroleum ether and successively extracted with methanol using soxhlet apparatus. The methanolic extract was evaporated to yield a dark brown solid (35 g), which was subjected to Si-gel CC (100-120 mesh) eluted with, ethyl acetate:methanol (9:1) to yield compound **AG-14** (247 mg, 0.705 % yield) and compound **AG-15** (268 mg, 0.69 % yield). The purity of eluted compound was tested using thin layer chromatography developed in solvent system of ethyl acetate:acetone (49:1) and ethyl acetate:methanol (9.9:0.1) for compounds **AG-14** and **AG-15**, respectively.

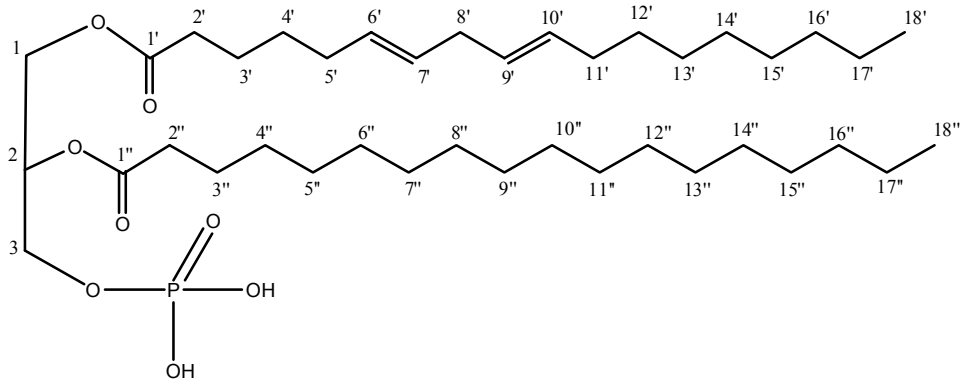
Compound AG-14: Linolein-2-stearin phosphate: Brown coloured semisolid mass, UV λ_{max} (MeOH) : 257 nm; IR (KBr, cm^{-1}): 3430, 1725, 1640, 1610, 1451, 1383, 1262, 1167, 1117, 828, 719; ^{1}H NMR (DMSO-d_6) δ: 6.70 (2H, m, H-7', H-9'), 5.32 (2H, m, H-6', H-10'), 3.72 (1H, m, C-2), 3.57 (2H, m, C-3), 3.11 (2H, m, C-1), 2.77 (2H, dd, *J* = 7.1, 7.1, C-2'), 2.55 (2H, d, *J* = 4.8 Hz, C-2''), 2.17 (2H, m, C-8''), 1.98 (2H, m, C-5'), 1.84 (2H, m, C-11'), 1.48 (2H, m, CH_2), 1.23 [44 H, brs, 22 x (CH_2)], 0.85 (3H, t, *J* = 6.1 Hz, C-18'), 0.82 (3H, t, *J* = 6.1 Hz, C-18''); ^{13}C NMR (DMSO-d_6): 174.45 (C-1'), 172.36 (C-1''), 129.63 (C-6'), 127.72 (C-7'), 115.76 (C-9'), 115.27 (C-10'), 76.52 (C-2), 70.59 (C-3), 63.19 (C-1), 55.52 (C-2'), 55.49 (C-2''), 33.65 (CH_2), 31.27 (CH_2), 28.99 (19 X CH_2), 28.68 (CH_2), 28.54 (CH_2), 26.57 (CH_2), 24.46 (CH_2), 22.07 (CH_2), 13.9 (CH_3-18' and CH_3-18''); FAB-MS m/z (Relative intensity): 700 $[M]^+$ ($C_{39}H_{73}O_8P$) (1.1), 356 (42.8), 267 (33.2), 263 (10.5).

Compound AG-15: Linolenic acid β-D-glucoside: Brown coloured semisolid mass, UV λ_{max} (MeOH): 256 nm; IR (KBr, cm^{-1}): 2921, 2851, 1712, 1642, 1509, 1453, 1383, 1263, 1112, 835, 721; ^{1}H NMR (DMSO-d_6) δ: 6.69 (2H, m, H-12,H-13), 5.28 (2H, m, H-10, H-15), 5.26 (2H, m, H-9, H-16), 4.63 (1H, d, *J* = 7.1 Hz, H-1'), 4.42 (1H, m, H-5'), 3.97 (1H, m, H-3'), 3.58 (1H, m, H-2'), 3.37 (1H, m, H-4'), 3.22 (1H, d, *J* = 11.3 Hz, H-6'a), 3.19 (1H, d, *J* = 11.3 Hz, H-6'b), 2.53 (2H, m, H-2), 2.49 (2H, m, H-11), 2.26 (2H, m, H-14), 2.14 (2H, m, H-8), 1.96 (2H, m, H-17), 1.47 (2H, m, CH_2), 1.20 [8H, brs, 4 x (CH_2)], 6.81(3H, t, *J* = 6.3 Hz, CH_3-18); ^{13}C NMR (DMSO-d_6): 174.44 (C-1), 129.65 (C-8), 129.51 (C-9), 127.73 (C-12), 127.03 (C-13), 115.91 (C-15), 115.38 (C-16), 105.63 (C-1'), 76.91 (C-5'), 73.51 (C-2'), 72.61 (C-3'), 70.74 (C-4'), 63.21 (C-6'), 55.63 (C-2), 51.45 (C-11), 33.77 (C-14), 31.53 (C-8), 31.08 (C-17), 29.33 (2 x CH_2), 28.85 (CH_2), 25.27 (CH_2), 22.28 (CH_2), 13.87 (Me-18); FAB-MS m/z (Relative intensity): 440$[M]^+$ ($C_{24}H_{40}O_7$) (1.1), 277 (10.3), 249 (21.6), 220 (71.5).

RESULTS AND DISCUSSION

Compound **AG 14**, designated as linolein-2-stearin phosphate was obtained as brown coloured semisolid mass from solvent system of ethyl acetate:methanol (9:1) as eluant. **AG-14** decolourized bromine water and produced foam on saponification. The IR (cm^{-1}) spectrum of **AG-14** exhibited characteristic absorption bands for hydroxyl group, ester, unsaturation and long aliphatic chain at 3430, 1725, 1451, 719 and 828.

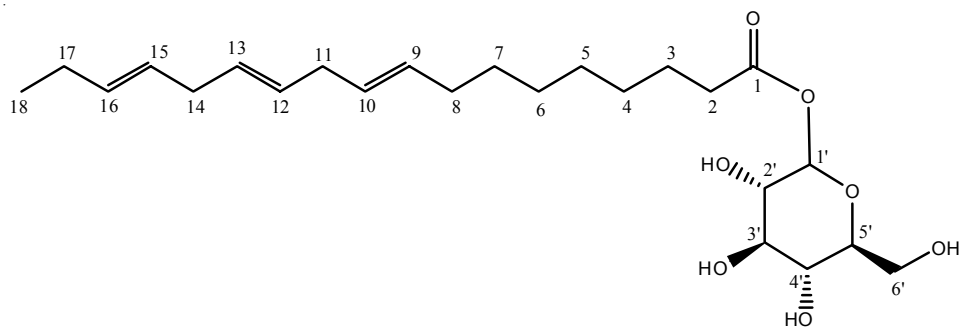
The mass spectrum of compound **AG-14** exhibited a molecular important ion peaks at m/z 700 corresponding to a molecular formula of glyceride phosphate ($C_{39}H_{73}O_8P$). The formation of the prominent fragments of m/z 267 $[CO(CH_2)_{16}CH_3]^+$ suggested presence of stearyl group at C-2. The ^{1}H NMR spectrum of compound **AG-14** displayed two multiplets at δ 6.70 and 5.32 corresponding to each two vinylic protons of C-7', C-9' and C-6', C-10', respectively. A 1 proton multiplet at δ 3.72 was ascribed for carbinol proton of C-2. Two multiplets at δ 3.57 and 3.11, accounted for 2 protons of each oxygenated methylene unit at C-3 and C-1, respectively. A 2 proton doublet of doublet at δ 2.77 (J = 7.1 Hz) and a two proton doublet at δ 2.55 (J = 4.8 Hz) were associated with the oxygenated methylene protons of C-2' and C-2", respectively. Two three proton triplets at δ 0.85 (J = 6.1 Hz) and 0.82 (J = 6.1 Hz) were accounted C-18' and C-18" primary methyl protons, respectively. The remaining methylene protons resonated at δ 2.17 (2H), δ 1.81 (2H), δ 1.48 (2H) and δ 1.23 (44H). The ^{13}C NMR spectrum of compound **AG-14** showed important signals for ester carbons at δ 174.45 (C-1') and 172.36 (C-1"). Vinylic carbons exhibited signals at δ 129.63 (C-6'), 127.72 (C-7'), 115.76 (C-9') and 115.27 (C-10'). The signals resonating at δ 13.9 was assigned to terminal methyl carbons C-18' and C-18". Acid hydrolysis of compound **AG-14** yielded stearic acid and linolein (TLC comparable). On the basis of forgoing discussions the structure of **AG-14** has been elucidated as glyceryl 1-linoleate-2-stearate phosphate.



3-(Phosphonooxy)-2-(stearoyloxy)propyl octadeca-6,9-dienoate

Compound **AG-15**, designated as linolenic acid β-D-glucoside was obtained as brown coloured semisolid mass from solvent system of ethyl acetate:methanol (9:1) as eluant. **AG-15** decolourized bromine water indicating unsaturated nature of the molecule. **AG-15** gave positive tests for glycosides. The IR (cm^{-1}) showed the characteristic absorption bands for carboxyate group, unsaturation and long (aliphatic) chain at 1712, 1642 and 835, 721, respectively. The mass spectrum of **AG-15** displayed a molecular ion peak at m/z 440 consistent to the molecular formula of an unsaturated

fatty acid glyceride ($C_{24}H_{40}O_7$). It indicated four double bond equivalents; three of them were adjusted to the vinylic linkages and one to the carboxylate group. The ^{1}H NMR spectrum showed the 3 (2 proton) multiplets at δ 6.69, 5.28 and 5.26 assigned to vinylic carbons of (C-12, C-13), (C-10, C-15) and (C-9, C-16) protons, respectively. A 1 proton doublet at δ 4.63 with coupling interaction of δ 7.1 Hz was ascribed to anomeric C-1'. Two (1 proton) doublets at δ 3.22 (J = 11.3 Hz) and δ 3.19 (J = 11.3 Hz) were attributed to oxygenated methylene protons. The remaining sugar protons resonated as multiplets between δ 4.42-3.37. A two proton multiplet at δ 2.53 was accounted C-2 methylene protons adjacent to the carboxylate ion. Four (2 proton) multiplets at δ 2.49, 2.26, 2.14 and 1.96 were associated with the methylene protons adjacent to the vinylic carbons. A three proton triplet at δ 0.81 (J = 6.3 Hz) was accounted for C-18 primary methyl protons. The remaining methylene protons signals appeared at δ 1.47 (2H) and δ 1.20 (8H). The ^{13}C NMR spectra of **AG-15** displayed signals for carboxylate carbon at δ 174.44 (C-1). The vinylic carbons exhibited their peaks at δ 129.65 (C-10), 129.51 (C-9), 127.73 (C-12), 127.03 (C-13), 115.91 (C-15) and 115.38 (C-16). The peaks at δ 13.87, 105.63, 76.91-63.21 and 55.63-22.28 corresponds to methyl carbon (C-18), anomeric carbon (C-1'), remaining sugar protons and methylene carbons. Acid hydrolysis of **AG-15** yielded linolenic acid and D-glucose. On the basis of these evidences the structure of **AG-15** has been formulated as octadeca-9,12,15-trienoic acid-β-D-glucopyranoside. (This compound is first time isolated and reported).



3,4,5-Trihydroxy-6-(hydroxymethyl)-tetrahydro-2*H*-pyran-2-yl)octadeca-9,12,15-trienoate

ACKNOWLEDGEMENTS

The authors acknowledge CDRI, Lucknow, IIT, Delhi and Arbro Pharmaceuticals for carrying out spectral analysis. Thanks are due to Dr. S.P. Basu, Director, Noida Institute of Engineering and Technology, Greater Noida for his valuable support and guidance.

REFERENCES

1. Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products, National Institute of Science Communication, CSIR, New Delhi, India (1997).
2. Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products, National Institute of Science Communication, CSIR, First Supplement Series (Raw Materials), New Delhi, India (2004).
3. L.V. Asolkar, K.K. Kakkar and O.J. Chakre, Second Supplement to Glossary of Indian Medical Plants with Active Principles, Part-I (A-K), pp. 50-51, 1965-1981 (1992).
4. N.D. Prajapati, S.S. Purohit, A.K. Sharma, A Handbook of Medicinal Plants, Agrobios India, Jodhpur (2004).
5. R.P. Rastogi and B.N. Mehrotra, Compendium of Indian Medicinal Plants, National Institution of Science Communication, CSIR, New Delhi, Vol. 2, pp. 1970-1979 (2006).
6. V.K. Raina, S.K. Srivastava and K.V. Syamasunder, *Flav. Fragrance J.*, **17**, 358 (2002).
7. B.R. Barik, A.B. Kundu and A.K. Dey, *Phytochemistry*, **26**, 2126 (1987).
8. J. Oonmetta-Aree, T. Suzuki, P. Gasaluck and G. Eumkeb, *LWT Food Sci. Technol.*, **39**, 1214 (2006).
9. T. Juntachote and E. Berghofer, *Food Chem.*, **92**, 193 (2005).
10. A.M. Janssen and J.J.C. Scheffer, *Planta Med.*, 507 (1985).
11. C.C. Lee and P. Houghton, *J. Ethnopharmacol.*, **100**, 237 (2005).
12. H. Matsuda, Y. Pongpiriyadacha, T. Morikawa, M. Ochi and M. Yoshikawa, *Eur. J. Pharmacol.*, **471**, 59 (2005).
13. E. Stal, Thin Layer Chromatography, Academic Press, New York (1965).
14. J.B. Harborne, Phytochemical Methods-A Guide to Modern Technique of Plant Analysis, Springer International Limited, edn. 3 (1998).
15. H. Wagner and S. Bladt, Plant Drug Analysis-A Thin Layer Chromatography Atlas, CBS Publisher and Distributors, Delhi, India, edn. 2 (1995).

(*Received*: 16 August 2008; *Accepted*: 23 February 2009) AJC-7265