Chemistry of Natural Compounds, Vol. 45, No. 4, 2009

FATTY ACID COMPOSITION OF SEED OIL OF *Phlomis fruticosa* GROWING IN MONTENEGRO

M. D. Sokovic,[1] D. Dokovic,[2] V. Tesevic,[2] V. Vajs,[2] and P. D. Marin[3]*

UDC 547.916

Natural compounds with an allene structure (C=C=C) are very rare in plants. From the early literature data they are known to occur in seed oils of some species from the Lamiaceae family [1, 2]. One of these compounds was characterized as a fatty acid with 18 carbon atoms with a $\Delta^{5,6}$ *allene* structure and named laballenic acid because it was found in Labiatae (Lamiaceae) species [1].

Later investigations of seed oil fatty acids confirmed the presence of laballenic acid in a number of species belonging to the Lamioideae subfamily [3–5]. According to available literature data, this unusual fatty acid is not found anywhere else in the plant kingdom.

In the Lamiaceae species a number of unusual fatty acids were reported. Thus, lamenallenic acid was isolated and identified from *Lamium purpureum* L. [6, 7]. This acid seems to be restricted only to *Lamium* genus, since it has not been found in any other genus of the Lamiaceae. In addition to the mentioned acids, in more recent works several other allenic acids were reported [3, 8].

Phlomic acid (20:2 $\Delta^{7,8}$ *allene*) was discovered and described as a minor additional allenic fatty acid occurring in some species of the subfamily Lamioideae. This fatty acid has been found in several genera from the Lamioideae (*Lamium, Ballota, Galeopsis, Stachys, Leonurus*, etc.) [8].

From the *Phlomis* genus, several species have been analyzed so far [1, 7–9]. Laballenic acid was found in all investigated species.

In this paper the fatty acid composition of seeds (nutlets) of *Phlomis fruticosa* was analyzed using GC and GC/MS. The following usual fatty acids are identified: myristic (14:0), palmitic (16:0), stearic (18:0), oleic (18:1 Δ^9), and linoleic (18:2 $\Delta^{9,12}$) acids, and two unusual fatty acids, laballenic (18:2 $\Delta^{5,6}$) and phlomic (20:2 $\Delta^{7,8}$) acids.

After esterification of total lipids, IR spectra were recorded. According to this analysis the presence of the allenic (ν (C=C=C)), ~1900 cm^{-1} and the carbonyl structure (ν (C=O)), ~1746 cm^{-1}, which are characteristic of unusual fatty acids, was proved.

According to GC/MS spectra, the series of fragments (*m/z* 94, 121, and 154), is a result of splitting of the molecule. These fragments are characteristic for 18:2 $\Delta^{5,6}$ allenic acid (laballenic acid). This acid is one of the dominant fatty acids in seed oils of this species. Comparing the results of GC/MS of our sample and literature data, it can be seen that ion *m/z* 94, which is specific for allene structures, was also one of the dominant.

It can be presumed that during the experiment phlomic acid was degraded. This acid was present in traces in this species. The presence of this acid correlates with the occurrence of another unusual fatty acid, 20:1 Δ^9*cis* or 20:1 n-11. The combination of these two fatty acids is expected, since phlomic acid seems to be a chain-elongation product of laballenic acid [3].

According to literature data it is evident that the presence of allenic fatty acids is a highly characteristic feature of the Lamioideae subfamily. Our results are in agreement with literature data. The chemotaxonomic significance of the presence or absence of phlomic acid in the Lamioideae is not yet known. Phlomic acid has been found in a number of genera from the Lamioideae subfamily (*Phlomis, Leonurus, Marrubium, Ballota, Galeopsis, Stachys*, etc.) where the seed oils contain also

1) Institute for Biological Research "Sinisa Stankovic", Bulevar Despota Stefana 142, 11000 Belgrade, Serbia; 2) University of Belgrade, Faculty of Chemistry, Studentski trg 16, P.O. Box 158, 11000 Belgrade, Serbia; 3) University of Belgrade, Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac", Studentski trg 16, 11000 Belgrade, Serbia, e-mail: pdmarin@bfbot.bg.ac.yu. Published in Khimiya Prirodnykh Soedinenii, No. 4, pp. 449–450, July–August, 2009. Original article submitted November 14, 2007.

 0009-3130/09/4504-0528

laballenic acid. It was found, however, in one sample of *Lamiastrum galeobdolon* but not in *Lamium maculatum* [6]. The presence of phlomic acid can be of significance as a taxonomic marker in distinguishing *Lamium* and *Lamiastrum* (which is sometimes included into *Lamium* genus in literature). In addition, lamenallenic acid, which is characteristic for *Lamium* genus, has not been detected in *Lamiastrum galeobdolon* [6].

Plant Material. *Phlomis fruticosa* was collected at Voluvica hill in September 2006, Bar, Montenegro. A voucher sample of this species was deposited in the Herbarium, Institute of Botany and Botanical Garden "Jevremovac" of the Faculty of Biology, University of Belgrade (BEOU).

Isolation of Fatty Acids. Seeds (nutlets) were separated from plant material , and the purity of the seeds was checked under a microscope; then the seeds were crushed in a mortar. 100 mg of nutlets of *Phlomis fruticosa* was dissolved in 1 mL of 2-propanol and the sample was boiled for 10 min at 80°C. The sample was then homogenized with 8 mL of BHT (butylated hydroxytoluene) (conc. 0.8 mg/mL MeOH) and used as an internal standard. The homogenized sample was stored at 4°C for 24 h for lipid extraction. Then 2 mL 0.7% NaCl in H_2O was added. The lower phase was taken from the funnel and transferred into a test tube and dried with anhydrous Na_2SO_4. The sample was evaporated and 6 mL of a mixture of 1% H_2SO_4 in MeOH was added. The sample was then heated for 2 h. A saturated solution of $NaHCO_3$ was added to the sample. After neutralization, the sample was extracted with methylene chloride (CH_2Cl_2), and the lower phase from the funnel was taken for further analysis.

IR spectra were recorded with a Perkin–Elmer 1725 X instrument. Qualitative data were determined by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS).

Gas Chromatography/Mass Spectrometry (GC/MS). A Varian model 3400 chromatograph, equipped with a split/splitless injector (250°C) and a SUPWAX fused silica capillary column 60 m × 0.32 mm and FID (300°C), was used for GC and GC/MS measurements. The column was temperature programmed as follows: 200–230°C, 2°C/min, carrier gas 3 mL H_2/min. Temperature of injector (SPLIT 1:1000) 250°C, temperature of detector 300°C. Peak areas were calculated electronically by a Varian DS-604 data station. GC/MS: the gas chromatograph was connected via an open split interface and a fused silica capillary (at 250° C) to the ion source of a Finnigan MAT 8230 mass spectrometer. Working conditions: carier gas 2 mL He/min; other GC conditions as above. MS: ion source (electron impact) 170°C, 70 eV.

ACKNOWLEDGMENT

The authors are grateful to the Ministry of Science of the Republic of Serbia for financial support (Grants Nos. 143041 and 143049).

REFERENCES

1. M. O. Bagby, C. R. Smith, and I. A. Wolff, *J. Org. Chem*., **30**, 4227 (1965).
2. J. M. Hagemann, F. R. Earle, I. A. Wolff, and A. S. Barclay, *Lipids*, **2**, 371 (1967).
3. K. Aitzetmuller, *Lamiales Newsletter (Kew/London)*, **5**, 3 (1997).
4. K. Aitzetmuller, N. Tsevegsuren, and K. Vosmann, *Fett-Lipid*, **99**, 74 (1997).
5. N. Tsevegsuren, K. Aitzetmuller, and G. Werner, *Lamiales Newsletter (Kew/London)*, **5**, 6 (1997).
6. K. L. Mikolajczak, M. F. Rogers, C. R. Smith, and I. A. Wolff, *Biochem. J.*, **105**, 1245 (1967).
7. D. T. Asilbekova, S. D. Gusakova, and A. U. Umarov, *Khim. Prir. Soedin*., 561 (1983).
8. K. Aitzetmuller and N. Tseveguren, *Lamiales Newsletter (Kew/London)*, **6**, 13 (1998).
9. K. Aitzetmuller, *J. High Resol. Chromatogr*., **16**, 488 (1993).