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SHORT REPORTS

UNSATURATED FATTY ACID FROM SEED OIL OF *CONSOLIDA REGALIS*

E. DABI, E. HÉTHELYI, I. ZÁMBÓ, P. TÉTÉNYI and V. SIMONIDESZ*

Research Institute for Medicinal Plants, Budakalász, Hungary; *Pharmaceutical Works, CHINOIN, Budapest, Hungary

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Key Word Index—*Consolida regalis*: Ranunculaceae; seed oil; fatty acids; icosenoic acid; HPLC.

Abstract—Icosenoic acid [20:1 (11)] was isolated from the seed oil of *Consolida regalis*. The seeds contain *ca* 30% of fatty oil which comprise *ca* 25% 20:1 (11). The structure of the fatty acid methyl ester having been isolated by column chromatography was determined by GC/MS, IR and ^{1}H NMR and was confirmed by HPLC analysis of the bromophenacyl ester derivative.

INTRODUCTION

Our research is concerned with the identification, isolation and analysis of unsaturated fatty acids with long carbon chains, to be found in the seed oils of Hungarian plants. According to published data [1–5] 5-icosenoic acid [20:1 (5)] occurs in a few species of the Ranunculaceae and Limnanthaceae. Because of these reports as well as our own earlier work [6], we expected to find *cis*-5-, or *cis*-11 20:1 as the main component of the seed oil of Hungarian *Consolida regalis* S. F. Gray (syn. *Delphinium consolida*).

In this paper we report the isolation and structural identification of the 20:1 acid from this plant.

RESULTS AND DISCUSSION

The seeds of *C. regalis* contain *ca* 30% fatty oil. The qualitative and quantitative composition of the fatty acid ester mixture obtained by saponification of the oil was determined by GC and GC/MS. The main acid is 18:1 (9), and the sample contained *ca* 25% 20:1 $[M]^+$ *m/z* 324. The position of the double bond could not be determined by direct comparison with authentic samples of 20:1 (*cis*-5) and (*cis*-11).

According to data published by Karrer [7] and Gibbs [8] the seed oil of *Simmondsia californica* and of *Delphinium* species contains 20:1 (11). For the purpose of further analyses the methyl ester of 20:1 was isolated from the ester mixture of *C. regalis* by means of CC. After GC/MS analysis, NMR and IR spectrometric analyses were also carried out on the isolated component. The mass spectrum of the component gave fragmentation characteristic of the 20:1 (11) methyl ester found in EPA/NIH [9] and different both from the 20:1 (5) spectrum obtained by the authors and from that published in the literature [10].

In the literature [11] descriptions of 20:1 (9), (14), (15) and (17) are also to be found, but with the exception of 20:1 (15) which Vickery [12] demonstrated in the seed oil of various members of the Proteaceae, the others occur in marine sources or in cod liver oil.

The ^{1}H NMR spectrum of the isolated component indicated that there was a double bond near the middle of the molecule, since here the localized hydrogens are bonded merely to the adjoining methylene groups.

According to the experiments of Durst *et al.* [13], Chang *et al.* [14], as well as those of our own, fatty acids of different unsaturation and of different carbon numbers, can be separated from each other by HPLC analysis of the corresponding bromophenacyl esters. We investigated separations by HPLC of the bromophenacyl esters of some unsaturated fatty acids, using two eluent systems (Table 1). Elution times of the components were considerably modified by very small changes of eluent compositions. By this means we succeeded in separating in

Table 1. HPLC retention times (min) of fatty acid bromophenacyl esters on a RP 18 column

Fatty acid	Eluent 90% MeOH	88% MeOH
18:3 (6, 9, 12)	4.92	—
18:2 (9, 12)	6.02	..
18:3 (9, 12, 15)	6.12	—
16:0	7.33	—
18:1 (9)	7.84	11.40
18:0	11.00	—
20:1 (*cis*-11)	11.41	—
20:1 (*cis*-5)	12.39	19.10
20:2 (*cis*-5, 13)	13.07	20.08
22:1	17.32	37.11
20:0	—	30.60

a selective manner different substances with nearly the same retention times. The R_t of the main component of the *C. regalis* seed oil was 11.78 min, which is in good agreement with the R_t of 20:1 (11.41 min). There is a substantial difference between the R_t of the isolated acid and that of 20:1 (*cis*-5) (12.39 min).

EXPERIMENTAL

Seed material originated from a commercial source. The oils from the seeds were obtained by petrol (40°) extraction for 8 hr in a Soxhlet apparatus. After evapn of solvent the oil obtained was saponified with KOH. The free fatty acids were esterified with MeOH–H_2SO_4. IR were recorded on KBr discs and NMR at 100 MHz in CCl_4. GC investigation of fatty acid Me esters were carried out using FID on a 2 m column of 3% DEGS using temp. programming.

GC/MS was carried at 70 eV out on a double focusing, EI ion source instrument with He carrier gas and 3% NPS and 3% DEGS columns. Analyses were conducted isothermally at 210° or programmed from 160 to 210° at 8°/min.

CC separation of the fatty acid Me ester mixture was achieved by gradual elution from a mixture of $AgNO_3$-impregnated silicic acid–celite (2:1, w/w) eluting with a mixture of *n*-hexane containing 25% C_6H_6 up to Et_2O.

The spectral data obtained by NMR and IR analyses were as follows: IR: ν_{max}^{KBr} cm^{-1}: 1744 (C=O), 1170 (C–O–C), 1020 (C–O–C). ^1H NMR: δ 3.68 (*s*, 3H, $-\overset{O}{\overset{\|}{C}}-O-CH$), 5.33 (*t*, 2H, C=C), 2.30 (*t*, 2H, $-CH_2$ COOMe, 2.06 (*m*, 4H, $-CH_2CH{=}CHCH_2-$, 1.66 (*m*, 2H, $-CH_2CH_2COOMe$), 1.33 (wide, 24H, CH_2) 0.92 (*t*, 3H, Me).

HPLC was performed on LiChrosorb RP 18 (10 μm), eluents being MeOH–H_2O (9:1 and 22:3), flow rate 2 ml/min, detection at 254 nm. Bromophenacyl esters were prepared from 0.02 mM solns of acids. To the soln in $CHCl_3$–MeOH (2:1) was added a 5-fold amount of K_2CO_3 and phenolphtalein indicator; the solvent was evapd at 50°. To the dry residue a 3-fold excess of an MeCN soln of esterifying agent was added (dibromoacetophenone–crown ether, 10:1). The reaction mixture was refluxed for 45 min at 80°. After cooling the solvent was evapd and the residue dissolved in $CHCl_3$ for HPLC injection.

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REFERENCES

1. Bhatty, M. K. and Craig, B. M. (1966) *Can. J. Biochem.* **44**, 311.
2. Smith, C. R., Bagby, M. O., Miwa, T. K., Lohmax, R. L. and Wolff, I. A. (1960) *J. Org. Chem.* **25**, 1770.
3. Wolff, I. A. (1966) *Science* **154**, 1440.
4. Gorjajev, M. J. and Evbagova, N. A. (1977) *Szuravoncsnyi po Gazozsidkosztnoj Kromatografij Organyicseszkij Kiszlot.* Alma Ata, Nauka.
5. Chisholm, M. J. and Hopkins, C. Y. (1966) *Can. J. Chem.* **34**, 459.
6. Dabi-Lengyel, E., Tétényi, P., Héthelyi, I., Simonides, V. and Dobos J. (1982) *Herba Hung.* **21**, 149.
7. Karrer, W. (1958) *Konstitution und Vorkommen der Organischen Pflanzenstoffe.* Birkhauser, Basel.
8. Gibbs, R. D. (1974) *Chemotaxonomy of Flowering Plants.* McGill-Queens University Press.
9. Heller, S. R. and Milne, G. W. A. (1978) *EPA/NIH Mass Spectral Data Base.* U.S. Government Printing Office, Washington.
10. Stenhagen, E., Abrahamsson, S. and and McLafferty, F. W. (1974) *Registry of Mass Spectral Data.* John Wiley, New York.
11. Buckingham, J. (1982) *Dictionary of Organic Compounds*, 5th edn. Chapman & Hall, New York.
12. Vickery, J. R. (1971) *Phytochemistry* **10**, 123.
13. Durst, H. D., Milano, E. I., Kikta, S. A. Jr. and Conelly, E. (1975) *Analyt. Chem.* **47**, 1797.
14. Chang, S.-P. and Rothfus, J. A. (1976) *Lipids* **11**, 814.