CPFA), respectively (were used as substrate. The CPFA content of the substrate and product was determined by the AOAC procedure [10]. The crude enzyme fraction was obtained by aqueous extraction of the dehulled air-dried and defatted (solvent system $CHCl_3$–MeOH, 2:1) germinating cottonseed. For this purpose, well developed delinted cottonseed of Hybrid-4 variety (*Gossypium hirsutum*) were allowed to germinate by keeping for 2 days between folds of wet filter paper, and the non-shell portion was air-dried and ground before defatting.

In a typical experiment, the defatted powder (10 g) was extracted with H_2O (20 ml) by vigorous agitation for 1 hr at room temp. The contents were centrifuged at $12000 \times g$ for 15 min. The supernatant (10 ml) and substrate (100 mg) were mixed and agitated in a shaker for a predetermined time at room temp. and the oily layer was extracted with hexane, washed with H_2O, dried over Na_2SO_4, filtered and the solvent removed. The residual oil was tested for CPFA content by the standard AOAC procedure [10], and also analysed by ^1H NMR. The recovery of the fatty portion after the enzyme treatment was over 90%.

Acknowledgements—We are grateful to Dr S. N. Pandey, (Director, Jute Technological Research Laboratories, Calcutta) for valuable discussion and criticism of the manuscript and to the Prof. J. G. Kane Memorial Trust, Bombay for financial support for this work.

REFERENCES

1. Johnson, A. R., Judith, A. P., Shenstone, F. S., Fogerty, A. C. and Giovanelli, J. (1965) *Lipids* **2**, 308.
2. Shenstone, F. A., Vickery, J. R. and Johnson, A. R. (1965) *J. Agric. Food Chem.* **13**, 410.
3. Hooper, N. K. and Law, J. H. (1965) *Biochem. Biophys. Res. Commun.* **18**, 426.
4. Smith, G. N. and Bul'Lock, J. D. (1964) *Biochem. Biophys. Res. Commun.* **17**, 433.
5. Pandey, S. S. (1987) Ph.D. thesis submitted to the University of Bombay.
6. Pandey, S. S. and Subrahmanyam, V. V. R. (1986), *J. Am. Oil Chem. Soc.* **63**, 268.
7. Hopkins, C. Y. and Bernstein, H. (1959) *Can. J. Chem.* **37**, 775.
8. Goldfine, H. J. (1964) *Biol. Chem.* **239**, 2130.
9. Longone, D. T. and Miller, A. H. (1967) *Chem. Commun.* 447.
10. *Official Methods of Analysis of the AOAC* (1974) 12th Edn, 28.099.
11. Kircher, H. W. (1964) *J. Am. Oil Chem. Soc.* **41**, 4.

Phytochemistry, Vol. 27, No. 12, pp. 3964–3965, 1988.
Printed in Great Britain.

0031-9422/88 $3.00 + 0.00

PALMITIC ACID ESTER OF SITOSTERYL 3β-GLUCOSIDE FROM *CENTAUREA REGIA*

A. ULUBELEN, S. ÖKSÜZ and A. H. MERIÇLI

Faculty of Pharmacy, University of Istanbul, Istanbul, Turkey

(*Received* 12 *April* 1988)

Key Word Index—*Centaurea regia*; Compositae; sitosteryl 3β-glucoside 6′-*O*-palmitate; steroids; triterpenes; flavones; phenolic compounds; lignan.

Abstract—A new steroid derivative, sitosteryl 3β-glucoside 6′-*O*-palmitate, together with sitosterol, sitosteryl 3β-glucoside, lupeol, taraxasterol, crysoeriol, vitexin, luteolin 3′-glucoside, *p*-hydroxybenzoic acid, vanillic acid, *p*-hydroxyacetophenone and a lignan, (−)-arctigenin, were isolated from the aerial parts of *Centaurea regia*.

INTRODUCTION

In our previous studies with *Centaurea* species we have obtained flavonoids from *C. urvillei* [1] and *C. inermis*, *C. kilea*, *C. virgata* [2] and guaianolides from *C. behen* [3], *C. kotschyii* [4]. In this first chemical investigation of *Centaurea regia* Boiss. in addition to a new steroid derivative, sitosteryl 3β-glucoside 6′-*O*-palmitate, we have obtained known flavonoids crysoeriol, vitexin and luteolin 3′-glucoside [5] and terpenic compounds lupeol, taraxasterol, sitosterol, sitosteryl 3β-glucoside, as well as phenolic compounds *p*-hydroxybenzoic acid, vanillic acid and *p*-hydroxyacetophenone and a lignan (−)-arctigenin [6, 7].

RESULTS AND DISCUSSION

The residue from ether–petrol extracts of the plant material was fractioned on a silica gel column. The compounds obtained from the column were further separated by preparative TLC. The structures of the known compounds were established by spectral methods and, except for (−)-arctigenin, by direct comparison with authentic samples. Identification of (−)-arctigenin was

established by comparing its spectral data to those given in the literature [6, 7].

In the IR spectrum of a new compound (**1**) the peaks at 1735 and 1250 cm^{-1} indicated the presence of an ester while the large peaks at 3400 cm^{-1} and 1080, 1060, 1020 cm^{-1} showed the presence of polyhydroxyl groups. In addition to typical peaks for sitosterol the ^1H NMR spectrum of **1** showed the peaks for a fatty acid ester; the terminal methyl was at δ 0.87 (*t*, $J = 7$ Hz), the $(-CH_2-)_n$ at 1.36 (*br s*) and the methylene attached to a carbonyl group was at 2.35 (*t*, $J = 7$ Hz). The peaks at δ 4.46 (1H, *dd*, $J = 5$ Hz and 12 Hz), 5.36 (1H, *br d*, $J = 6$ Hz) indicated the steroidal H-3 and H-5 respectively. Other peaks at δ 4.27 (2H, *br d*, $J = 13$ Hz, H-6′), 4.38 (2H, *br d*, $J = 8.5$ Hz, H-1′ and H-5′), 3.38 (1H, *dd*), 3.50 (1H, *dd*) and 3.56 (1H, *dd*) indicated H-2′, H-3′ and H-4′ of the sugar moiety of the molecule. Together with the IR spectrum this data showed the presence of sitosteryl 3β-glucoside. The mass spectrum did not yield a molecular ion but the base peak at *m/z* 397 $[C_{29}H_{49}]^+$ suggested the steroidal part, the peak at *m/z* 255 $[C_{16}H_{31}O_2]^+$ indicated the fatty acid portion of **1**. The $[M-C_{16}H_{32}O_2]^+$ ion was present at *m/z* 576. After alkaline hydrolysis of **1** the steroidal part was established as sitosterol 3β-glucoside (^1H NMR of its acetyl derivative, TLC comparison with authentic sample as well as acid hydrolysis). The aliphatic acid part of the molecule was esterified with diazomethane and identified by GC as palmitic acid.

EXPERIMENTAL

The plant material was collected from south-eastern Turkey in July 1987 and identified by one of us (A.H.M). A voucher specimen is deposited in the Herbarium of the Faculty of Pharmacy, University of Istanbul (ISTE 58102).

Air-dried and powdered aerial parts of *C. regia* (1.8 kg) were extracted with Et_2O–petrol (1:2) and the extract treated with MeOH to remove long chain saturated hydrocarbons, the residue was separated on a silica gel column. The fractions were further separated by preparative TLC plates (E. Merck) to yield crysoeriol (7 mg), vitexin (6 mg), luteolin 3′-glucoside (10 mg), sitosterol (15 mg), sitosteryl 3β-glucoside (50 mg), lupeol (5 mg), taraxasterol (5 mg), (−)-arctigenin (150 mg), *p*-hydroxybenzoic acid (10 mg), *P*-hydroxyacetophenone (10 mg), vanillic acid (13 mg) and sitosteryl 3β-glucoside 6′-*O*-palmitate (15 mg). Sitosteryl 3β-glucoside 6′-*O*-palmitate (**1**). Amorphous, colourless compound. IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3400, 1080, 1060, 1020 (OH), 1735, 1250 (ester CO), 1465, 1380, 1165, 1110, 755, 720 $(-CH_2-)_n$. ^1H NMR data given in the text. MS 70 eV (probe) *m/z* (rel. int.): 576 $[M-C_{16}H_{32}O_2]^+$ (0.3), 558 $[576-H_2O]^+$ (0.5), 526 $[558-H_2O-CH_2]^+$ (0.5), 509 $[526-Me]^+$ (0.5), 397 $[C_{29}H_{49}]^+$ (100), 382 $[397-Me]^+$ (17), 255 $[C_{16}H_{31}O_2]^+$ (18), 211 $[C_{15}H_{31}]^+$ (10), 83 (68), 57 (96).

Hydrolysis of **1**. Compound **1** (5 mg) was dissolved in $CHCl_3$ and refluxed with 5% KOH for 8 hr. After removing the sitosteryl 3β-glucoside, the fatty acid thus obtained was esterified with CH_2N_2 in C_6H_6. (−)-Arctigenin: white, crystalline compound, mp 101°, $[\alpha]_D^{20°}$ −38° (1% in $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 281 (3.80), 231 (4.20). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 3010, 2939, 2850, 1760, 1605, 1580, 1520, 1450, 1380, 1265, 1235, 1165, 1025, 860, 810, 760. ^1H NMR ($CDCl_3$) δ: 3.81 (6H, *s*, 2xOMe), 3.84 (3H, *s*, OMe), 6.45 (1H, *d*, $J = 2$ Hz, H-6), 6.54 (1H, *dd*, $J = 2$ Hz and 7.5 Hz, H-4), 6.73 (1H, *d*, $J = 7.5$ Hz, H-3), 6.63 (1H, *d*, $J = 2$ Hz, H-3′), 6.60 (1H, *dd*, $J = 2$ Hz and 7.5 Hz, H-5′), 6.81 (1H, *d*, $J = 7.5$ Hz, H-6′), 5.6 (1H, *br s*, C-1′ OH), 4.13 (2H, *dd*, $J = 7$ Hz and 11 Hz, H-11), 2.62 (2H, *dd*, $J = 6$ Hz and 13 Hz, H-7), 2.54 (2H, *dd*, $J = 7$ Hz and 15 Hz, H-8), 2.48 (1H, *dd*, $J = 8$ Hz, H-9), 2.60 (1H, *dddd*, $J = 6$ Hz, 12 Hz and 14 Hz, H-10). MS 70 eV (probe) *m/z* (rel. int.): 372 $[M]^+$ ($C_{21}H_{24}O_6$) (82), 235 $[M-137]^+$ (7), 151 $[C_9H_{11}O_2]^+$ (70), 137 $[C_8H_9O_2]^+$ (100), 122 $[C_7H_6O_2]^+$ (8), 107 $[C_6H_3O_2]^+$ (12).

Acknowledgements—We thank Prof. Dr F. Bohlmann (T. U. Berlin) for the ^1H NMR and mass spectra.

REFERENCES

1. Ulubelen, A., Öksüz, S. (1982) *J. Nat. Prod.* **45**, 373.
2. Öksüz, S., Ayyildiz, H. and Johansson, C. (1984) *J. Nat. Prod.* **47**, 902.
3. Öksüz, S., Ulubelen, A., Aynehchi, Y. and Wagner, H. (1982) *Phytochemistry* **21**, 2747.
4. Öksüz, S. and Pütün, E. (1983) *Phytochemistry* **22**, 2615.
5. Litvinenko, V. I. and Sergienko, T. A. (1965) *Khim. Prir. Soedin.* 137; *C. A.* **63**, 10233 c.
6. Suzuki, H., Lee, K-H., Haruna, M., Iida, T., Ito, K. and Huang, H-C. (1982) *Phytochemistry* **21**, 1824.
7. Inagaki, I., Hisada, S. and Nishibe, S. (1972) *Chem. Pharm. Bull.* **20**, 2710.