Chemistry of Natural Compounds, Vol. 46, No. 1, 2010

PHYTOECDYSTEROIDS FROM *Digitalis ciliata* AND *D. purpurea* LEAVES

L. N. Gvazava[1*] and V. S. Kukoladze[2]

UDC 547.926

Ecdysteroids are chemically polyhydroxysteroids that contain specific structural elements such as 2,3-diol and 14α-hydroxy-Δ^7,6-ketone groups and *cis*-fused rings A and B. These features enable them to be classified as a separate group of natural compounds [1].

Ecdysones exhibit a specific effect on insect metamorphosis processes. Thus, juvenile and molting hormones were discovered among this group of compounds [2, 3]. A third generation of insecticides that had excellent selectivity and lacked harmful environmental effects could be created by using these properties against insects [4, 5].

Herein we report results from a study of ecdysteroids isolated from mother liquors after separation of steroidal saponins and cardenolides, in particular acetyldigitoxin and digalen-neo from *Digitalis ciliata* [6] and *D. purpurea* [7], respectively.

Cardenolides were exhaustively extracted from raw material (1 kg air-dried *D. ciliata* leaves and 0.8 kg, *D. purpurea*). The aqueous mother liquors were extracted with *n*-BuOH and purified from ballast substances by aqueous NaOH (5%). The purified *n*-BuOH extracts were condensed. The resulting precipitates were separated by filtration and dried to afford total ecdysteroids (5.84 g, 0.584% yield, *D. ciliata* and 3.92 g, 0.49% yield, *D. purpurea*). These were separated over columns of silica gel (L 100/160 μm, Czech Rep.) and Al_2O_3. We used solvent systems $CHCl_3$:MeOH (15:1, 1; 9:1, 2; 4:1, 3) and $CHCl_3$:MeOH:H_2O (4:1:0.1, 4) [8].

Elution of total ecdysteroids from *D. ciliata* first by system 1 and then system 2 isolated polypodine B acetate and benzoate and polypodine B. Elution of the column by systems 3 and 4 produced 20-hydroxyecdysone acetate and benzoate and 20-hydroxyecdysone.

Elution of total ecdysteroids from *D. purpurea* first by system 3 and then system 4 produced 20-hydroxyecdysone acetate and 20-hydroxyecdysone. Rechromatography of the total over a column of Al_2O_3 using systems 2 and 3 isolated viticosterone E.

The compounds were identified by the following data.

Polypodine B, $C_{27}H_{44}O_8$, mp 251–252°C (acetone), $[\alpha]_D^{20}$ +92.2° (*c* 0.30, MeOH). UV spectrum: 245 nm (log ε 4.01). IR spectrum (KBr, ν, cm^{-1}): 3350–3450, 1687 (C=O), 1640. MS *m*/*z* 478 $[M - H_2O]^+$. PMR spectrum (C_5D_5N, δ, ppm): 6.23 (1H, s, H-7), 4.0 –4.3 (2H, H-2,3), 3.85 (H-22), 3.59 (H-9), 1.58 (CH_3-21), 1.37 (6H, CH_3-26,27), 1.20 (CH_3-18), 1.15 (CH_3-19) [9, 10].

Polypodine B-22-*O*-acetate, $C_{29}H_{46}O_9$, mp 149 –152°C (MeOH:H_2O), $[\alpha]_D^{20}$ +122° (*c* 0.28, MeOH). IR spectrum (KBr, ν, cm^{-1}): 3400–3500 (OH), 1685 (C=O), 1735, 1260 (ester). MS *m*/*z* 520 $[M - H_2O]^+$. PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 6.25 (1H, br.s, H-7), 5.49 (1H, d, J = 7.6, H-22), 4.10 (2H, m, H-2, H-3), 3.62 (1H, m, H-9), 2.02 (3H, s, OAc), CH_3: 1.65, 1.35, 1.18 (×2) [8].

Polypodine B-22-*O*-benzoate, $C_{34}H_{48}O_9$, mp 196 –198°C (MeOH:H_2O). IR spectrum (KBr, ν, cm^{-1}): 3430 (OH), 1655 (C=O), 1710, 1280 (ester), 1612, 1585, 715 (arom.). PMR spectrum (C_5D_5N, δ, ppm, J/Hz): 8.24 (2H, dd, J = 1.2, 7.6) and 7.35 (3H, br.m), aromatic protons; 6.17 (1H, br.s, H-7), 5.70 (1H, d, J = 7.5, H-22), 4.16 (2H, m, H-2, H-3), 3.52 (1H, m, H-9), CH_3: 1.65, 1.17 (×2), 1.06, 1.02 [11].

20-Hydroxyecdysone, $C_{27}H_{44}O_7$, mp 242 –243°C (acetone), $[\alpha]_D^{20}$ +62.0° (*c* 1.4, MeOH). UV spectrum: 243 nm (log ε 4.05). IR spectrum (KBr, ν, cm^{-1}): 3500 –3350 (OH), 1660(Δ^7-6-ketone). MS *m*/*z* 462 $[M - H_2O]^+$. PMR spectrum

1) Kutateladze Institute of Pharmaceutical Chemistry, 0159, Tbilisi, ul. P. Saradzhishvili, 36, Georgia, e-mail: liligvazava@yahoo.com; 2) P. Melikishvili Institute of Physical and Organic Chemistry, 0186, Tbilisi, ul. Dzhikia, 5, Georgia.
Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 124–125, January–February, 2010. Original article submitted June 17, 2009.

0009-3130/10/4601-0146

(C_5D_5N, δ, ppm): 6.21 (1H, br.s, H-7), 4.1–4.4 (2H, m, H-2, H-3), 3.86 (1H, m, H-22), 3.54 (1H, m, H-9), 1.56 (3H, s, CH_3-21), 1.34 (6H, s, CH_3-26,27), 1.19 (3H, s, CH_3-18), 1.03 (3H, s, CH_3-19) [8, 12].

20-Hydroxyecdysone-22-*O*-acetate, $C_{29}H_{46}O_8$, mp 151 –154°C (acetone), $[\alpha]_D^{20}$ +92.0° (*c* 0.30, MeOH). IR spectrum (KBr, ν, cm^{-1}): 3400-3500 (OH), 1685 (C=O), 1660 (Δ^7-6-ketone), 1735, 1260 (ester). PMR spectrum (C_5D_5N, δ, ppm): 6.24 (1H, br.s, H-7), 5.52 (1H, m, H-22), 4.22 (2H, m, H-2, H-3), 3.56 (1H, m, H-9), 1.60 (3H, s, CH_3-21), 1.32 (6H, s, CH_3-26,27), 1.14 (3H, s, CH_3-18), 1.07 (3H, s, CH_3-19) [8].

20-Hydroxyecdysone-22-*O*-benzoate, $C_{34}H_{48}O_8$, mp 202 –204°C (MeOH:H_2O), $[\alpha]_D^{20}$ +45.8° (*c* 1.1, MeOH). UV spectrum: 235 nm (log ε 4.36). IR spectrum (KBr, ν, cm^{-1}): 3420–3480 (OH), 1660 (Δ^7-6-ketone), 1710, 1285 (ester), 1610, 1587, 720 (benzene). MS *m*/*z* 584 $[M]^+$. Proton spectrum was analogous to that published [13].

Viticosterone E, $C_{29}H_{46}O_8$, mp 196 –198°C (acetone), $[\alpha]_D^{20}$ +59.0° (*c* 0.74, MeOH). IR spectrum (KBr, ν, cm^{-1}): 3400 (OH), 1670 (Δ^7-6-ketone), 1730, 1275 (ester). PMR spectrum was identical to that published for viticosterone E [14].

All studied ecdysteroids were isolated and characterized from plants of the genus *Digitalis* for the first time.

REFERENCES

1. N. V. Kovganko, Zh. N. Kashtan, Yu. G. Chernov, S. K. Ananich, S. N. Sokolov, and V. L. Survilo, *Khim. Prir. Soedin.*, 335 (2003).
2. N. A. Tamarina, *Usp. Sovrem. Biol.*, **62**, 415 (1966).
3. E. A. Pridantseva, A. A. Drabkina, and Yu. S. Tsizin, *Usp. Soverm. Biol.*, **71**, 203 (1971).
4. J. E. Wright, W. T. Chamberlain, and C. C. Barrett, *Science*, **172**, 1247 (1971).
5. M. Sakai, Y. Sato, and S. Imai, *Insecticides*, Jpn. Pat., Cl 30F 352 (A 01n), No. 24264 (1971).
6. L. N. Gvazava and E. P. Kemertelidze, Pat. No. 611,442 (1978); *Byull. Izobret.*, No. 2,394,451 (1978).
7. E. P. Kemertelidze, *Soobshch. Akad. Nauk Gruz. SSR*, **25**, 285 (1960).
8. Z. Saatov, R. U. Umarova, M. B. Gorovits, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 480 (1990).
9. J. Jizba, V. Herout, and F. Sorm, *Tetrahedron Lett.*, 5139 (1967).
10. N. Sh. Ramazanov, E. S. Maksimov, Z. Saatov, A. U. Mamatkhanov, and N. D. Abdullaev, *Khim. Prir. Soedin.*, 392 (1997).
11. Z. Sadykov, N. Sh. Ramazanov, and Z. Saatov, *Khim. Prir. Soedin.*, 851 (1997).
12. N. Sh. Ramazanov, E. S. Maksimov, Z. Saatov, and N. D. Abdullaev, *Khim. Prir. Soedin.*, 395 (1997).
13. Z. Saatov, M. B. Gorovits, S. Melibaev, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 77 (1986).
14. I. L. Novosel′skaya, M. B. Gorovits, and N. K. Abubakirov, *Khim. Prir. Soedin.*, 668 (1981).