off, the residue was distilled, and a colorless mobile liquid was obtained giving on TLC in the petroleum ether—diethyl ether (1:1) system two spots with R_f 0.70 and 0.62.

The mixture was chromatographed on silica gel. Petroleum ether eluates yielded 0.7 g of a colorless mobile liquid with n_D^{20} 1.4480. IR spectrum (in $CHCl_3$), ν_{max}, cm^{-1}: 1380, 1455, 2880, 2940 (CH_3—C; CH_2—C). Mass spectrum: mixture of hydrocarbons with M^+ 204 ($C_{15}H_{24}$) and M^+ 206 ($C_{15}H_{26}$). The NMR spectrum also showed the presence of a mixture of these substances: broadened singlet at 1.7 ppm (CH_3—C=C); four singlets at 0.85, 0.9, 1.0, 1.1 (four quaternary methyls), doublets at 0.8-1.0 ppm.

Petroleum ether—diethyl ether (1:1) eluted 0.15 g of (II) in the form of a viscous liquid; IR spectrum (in $CHCl_3$), ν_{max}, cm^{-1}: 1742 (OCO), 1726 (C=O), 1370, 1455, 2870, 2950 (CH_3—C; CH_2—C). Mass spectrum: M^+ 280. The NMR spectrum is given above.

SUMMARY

From a carbon dioxide extract and also from the essential oil of the leaves of *Ledum palustre* L. the sesquiterpene ketone cyclocolorenone, hitherto unreported for this medicinal plant, has been isolated. The structure proposed for it previously has been confirmed by NMR and mass spectroscopy and also by the production of hydrogenation products.

LITERATURE CITED

1. N. P. Kir'yalov, Zh. Obshch. Khim., 21, No. 10, 2074 (1951).
2. L. Dolejs, O. Motl, M. Souček et al., Collection Czech. Chem. Commun., 25, 1483 (1960).
3. R. E. Corbett and R. N. Speden, J. Chem. Soc., No. 10, 3710 (1958).
4. P. I. Zakharov, P. B. Terent'ev, O. A. Konovalova, and K. S. Rybalko, Khim. Prirodn. Soedin., 344 (1977).
5. M. Schantz and R. Hiltunen, Scietia Pharmaceutica, No. 3, 137 (1971).
6. M. Schantz, K.-G. Wilden, and R. Hiltunen, Acta Chem. Scand., 27, 551 (1973).

PHYTOECDYSONES OF *Ajuga turkestanica*

VI. 22-ACETYLCYASTERONE

B. Z. Usmanov, Ya. V. Rashkes,
and N. K. Abubakirov

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Continuing a study of the phytoecdysones of *Ajuga turkestanica* (Rgl.) Briq. (family Labiatae), in addition to cyasterone, ecdysterone, turkesterone [1], ajugalacterone, and ajugasterone-B [2], which have been isolated previously, we have detected a new phytoecdysone. After the determination of its structure, it has been given the name of 22-acetylcyasterone (I).

The ecdysone from *A. turkestanica* has the elementary composition $C_{31}H_{46}O_9$. The UV spectrum of this compound contains an intense maximum at 240 nm (log ε 4.07), which is characteristic for Δ^7-6-oxo steroids. In the IR spectrum, in addition to absorption bands at 3440 cm^{-1} (OH), 1745 cm^{-1} (γ-lactone), and 1655 cm^{-1} (conjugated ketone), which are close to those for ecdysones for the cyasterone type [3, 4], bands are also observed at 1755 and 1255 cm^{-1} showing the presence of an ester grouping.

Quantitative oxidation of the new ecdysone with sodium periodate showed that its molecule includes one free diol group.

The ORD curve of compound (I) shows a positive Cotton effect: $[M]_{361}$ +2822°; $[M]_{260}$ —2515°; $a = +53°$. On the CD curve, Cotton effects are observed at 340 nm (Δε = +1.27) and 288 nm (Δε = —0.66).

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The PMR spectrum of the phytoecdysone (I) has signals from five methyl groups: singlets at 0.95, 1.06, and 1.47 ppm corresponding to the C-18, -19, and -21 C-methyls, respectively, and doublets at 1.21 and 1.32 ppm corresponding to the analogous C-27 and -29 groups. A comparison of the spectra of cyasterone and the phytoecdysone (I) showed that the most characteristic feature of the second compound is the presence of a three-proton singlet at 1.94 ppm, which is typical for the protons of an acetyl group, and a broadened one-proton signal at 5.32 ppm corresponding to a proton geminal to a secondary acetyl group. In addition to this, the spectrum of the new ecdysone derivative has the following signals the assignment of which causes no difficulties: 4.05 ppm (s, 2H, H-2, 3), 3.44 ppm (m, 1H, H-9), 3.90 ppm (m, 1H, H-28), and 6.12 ppm (s, 1H, H-7). The singlets and multiplets listed have a broadened form. The signals from the hydroxy groups, also in the form of a broadened elevation, occupy the part of the spectrum between 4.5 and 6.5 ppm.

The peak of the ion with the highest mass number observed in the mass spectrum of (I) contains 544 m.u. The presence of the peaks of ions with m/e 183 and 139 shows that compound (I) belongs to the C-29 ecdysones with a lactone ring [3, 4] and with hydroxy functions at C-20 and C-22.

The acetylation of (I) with acetic anhydride formed a diacetate (II) (M^+ 646), the IR spectrum of which coincided with that of cyasterone 2β,3β,22-triacetate.

In dry acetone solution in the presence of molybdophosphoric acid, the ecdysone (I) formed a monoacetonide (III) (M^+ 602). Consequently, the molecular weight of the initial natural compound (I) is 562 and the ion with m/e 544 in its spectrum represents the fragment $(M - H_2O)^+$.

The next problem consisted in establishing the position of the acetoxy group. It appeared most likely that this group was present at C_{22}. A confirmation of this was the suppression in the mass spectrum of (I) of decomposition at the C-20—C-22 bond that is characteristic of ecdysones with a diol group in the corresponding positions. The peaks of the ions with m/e 363, 345, and 327 that are characteristic for ecdysones with the ecdysterone nucleus, including cyasterone [3, 4] have a low intensity in the spectrum of the new phytoecdysone (I).

On the other hand, in the mass spectra of substances (I-III) a series of directions of fragmentation that are not characteristic for cyasterone are observed. These are obviously due to the presence of an acetoxy group at C-22 (Scheme). One of the varients of this group in the form of the CH_3COOH molecule, leading to the ejection of a stabilized allyl radical with 125 a.m.u., gives oxonium fragments with m/e 377 (I), 461 (II), and 417 (III). These fragments, in their turn, by losing H_2O, are converted into the ions most common in the middle part of the spectrum with m/e 359 (I), 443 (II), and 399 (III).

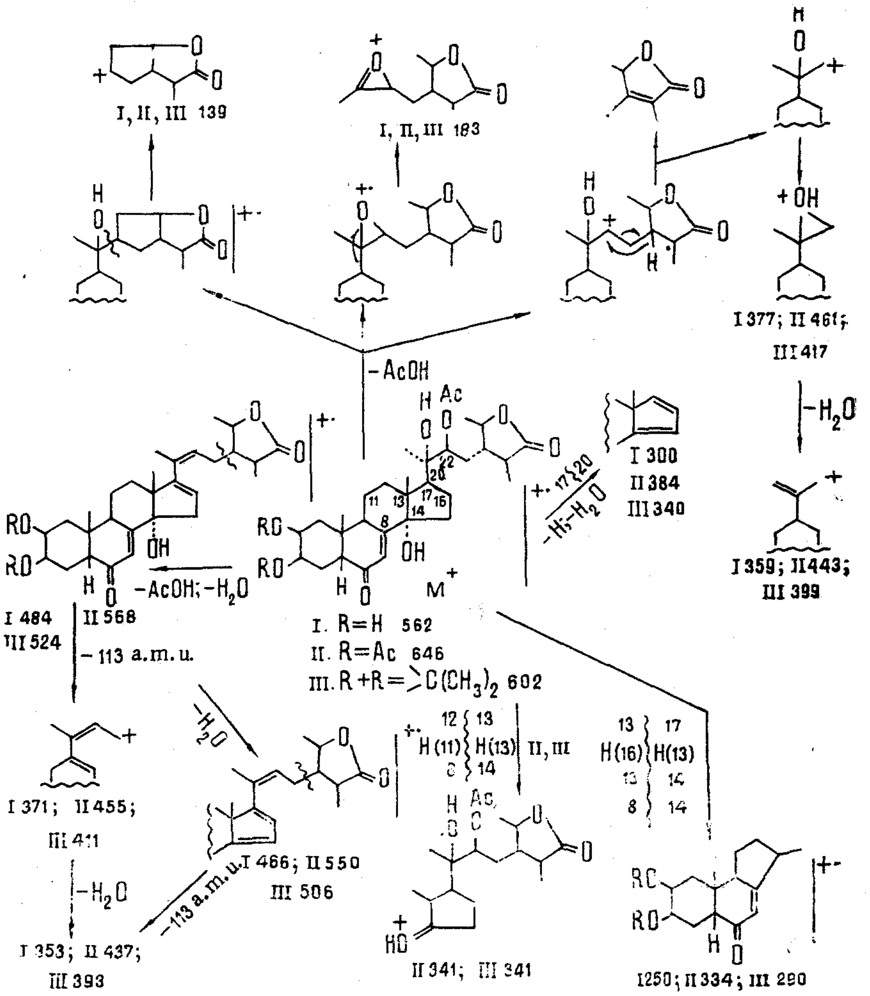
The alternative elimination of AcOH and H_2O leads to the appearance of a conjugated chain promoting the cleavage of the C-22—C-24 bond with the formation of allyl cations having m/e 371 and 353 (I), 455 and 437 (II), and 411 and 393 (III). Cleavage of the C-23—C-24 bond may also take place after the previous splitting out of H_2O through intermediate ions with m/e 466 (I), 550 (II), and 506 (III).

In the spectra of the diacetate (II) and the acetonide (III) we observed intensive fragments with the same mass number of 341. In the spectrum of the DO analog of the diacetate (II), this peak was shifted by 2 m.u. This fact confirms the inclusion in the ion under consideration of the whole of the side chain and of ring D.

The ion with m/e 183 in the spectra of the DO analogs of (I) and (II) does not contain the isotopic label, which indicates that it was formed from the ion $(M - AcOH)^+$ having the structure of an α-oxide. The absence of additional substituents in the steroid nucleus of the new phytoecdysone (I) is confirmed by the fact that its spectrum, like the spectrum of cyasterone [3, 4] contains the peak of an ion with m/e 300 characterizing the cleavage of the C-17—C-20 bond with the simultaneous elimination of H_2O at the expense of the hydroxyl at C-14.

In the spectra of (II) and (III), this peak is shifted in the direction of high masses by 84 and 40 m.u., respectively. The presence of only two oxygen-containing groups in rings A-C is shown by the mass numbers of the ions with m/e 250 (I), 334 (II), and 290 (III). They have structures close to the structures of the main characteristic ions in the spectra of the cardenolides [5, 6].

The facts given show that the new phytoecdysone in fact has the structure of 22-acetylcyasterone.



Fragmentation of 22-acetylcyasterone (I) and its 2,3-diacetate (II) and 2,3-acetonide (III)

EXPERIMENTAL

For chromatography we used type KSK silica gel treated in the usual way and Czech type "L" alumina (activity grade IV). The purity of the substances was checked by thin-layer chromatography on KSK silica gel in the chloroform–ethanol (4:1, 9:1, and 25:1) systems. The phytoecdysones were revealed with vanillin–sulfuric acid.

The IR spectra were taken on a UR-20 spectrometer (KBR), the PMR spectra on a JMN-4H-100 instrument (δ scale, 0 — HMDS), and the mass spectra on a MKh-1303 instrument fitted with a system for the direct introduction of the substances into the ion source, at an ionizing voltage of 40 V and a temperature of 140-200°C. Deuterium exchange of compound (I) and (II) was effected with the aid of CD_3OD.

Isolation of 22-Acetylcyasterone (I). The air-dry leaves of *A. turkestanica* (1.1 kg) were extracted with 6 liters of methanol. The extract was concentrated, diluted with water, and treated with hexane. Then the aqueous layer was exhaustively extracted with ethyl acetate. After the solvent had been distilled off, 26.7 g of a dry pulverulent extract was obtained. This combined material was chromatographed on a column of alumina (1 kg). Elution was performed first with chloroform and then with chloroform–ethanol (9:1). The partially purified combined material was rechromatographed on a column of silica gel. Elution with chloroform containing 2, 3, 4, 5, and 10% of ethanol yielded successively ajugalactone, ajugasterone-B, cyasterone, and the ecdysone (I), and, on further elution, ecdysterone. The yield of the new ecdysone was 0.547 g (0.05% of the weight of the air-dry raw material).

22-Acetylcyasterone (I), $C_{31}H_{46}O_9$, mp 212-215°C (acetone–hexane); $[\alpha]_D^{29}$ +96.0 ± 2° (c 0.56; methanol); R_f 0.35 [chloroform–ethanol (9:1) system on silica gel]. It was readily soluble in ethanol, ethyl acetate, and acetone, moderately soluble in ether, and sparingly soluble in chloroform. Mass spectrum, m/e (%): 544 ($M - H_2O$; 1), 526 ($M - 2H_2O$; 2), 516 ($M - H_2O–CO$; 1), 508 ($M - 3H_2O$; 1), 498 ($M - 2H_2O - CO$; 12), 484 ($M - AcOH - H_2O$; 16), 466 ($M - AcOH - 2H_2O$; 85), 456 (16), 451 (14), 448 (8), 433 (11), 377 (5), 371 (12), 363 (3), 360 (10), 359 (16), 353 (10), 345 (5), 327 (8), 301 (10), 300 (13), 299 (11), 250 (10), 249 (11), 183 (22), 139 (19), 69 (42), 60 (14), 43 (100).

22-Acetylcyasterone 2,3-diacetate (II) from (I). The ecdysone (I) (130 mg) in 1.3 ml of pyridine was acetylated with 0.65 ml of acetic anhydride at room temperature for 20 h. The excess of reagents was driven off in vacuum. The residue (146 mg) was chromatographed on silica gel (25 g). Elution of the column with chloroform–ethanol (100:1) yielded 105 mg of product (II), $C_{35}H_{50}O_{11}$, mp 248-249°C (from methanol); $[\alpha]_D^{29}$ +72.4 ± 2° (c 0.48; methanol); $\lambda_{max}^{C_2H_5OH}$ 242 nm (log ε 4.03); ν_{max}^{KBr}, cm^{-1}: 3470 (OH), 1770, 1740 (C=O of a lactone and of acetyl groups), 1663 (conjugated ketone), and 1250 (ester). Literature information [4] for 2,3,22-triacetylcyasterone: mp 251-252°C; $[\alpha]_D$ +69.0° (c 2.6); $\lambda_{max}^{C_2H_5OH}$ 243 nm (log ε 4.11); ν_{max}^{KBr}, cm^{-1}: 3480, 1776, 1742, 1657, 1242.

Mass spectrum of (II), m/e (%): 646 (M^+; 1), 628 ($M - H_2O$; 3), 618 ($M - CO$); 4), 610 ($M - 2H_2O$; 3), 600 (2), 586 ($M - AcOH$; 3), 582 (6), 568 (49), 558 (10), 550 (52), 540 (94), 535 (13), 526 (32), 498 (11), 480 (6), 466 (23), 456 (28), 455 (56), 448 (31), 444 (40), 443 (52), 437 (40), 433 (28), 384 (24), 383 (17), 341 (42), 334 (78), 333 (45), 183 (38), 139 (21), 69 (42), 60 (16), 43 (100).

22-Acetylcyasterone 2,3-Acetonide (III) from (I). A solution of 67.8 mg of the ecdysone (I) in 10 ml of anhydrous acetone was treated with 3 mg of molybdophosphoric acid, and the reaction was left at room temperature for 25 h. Then the solution was concentrated in vacuum to half its original volume and was diluted with water and neutralized with sodium bicarbonate solution. The neutral solution was extracted with ethyl acetate and the extract was chromatographed on a column of silica gel (10 g). Elution of the column with chloroform–ethanol (100:1) gave 27 mg of the amorphous acetonide (III), $C_{34}H_{50}O_9$; $\lambda_{max}^{C_2H_5OH}$ 238 nm (log ε 4.14); ν_{max}^{KBr}, cm^{-1}: 3470, 1770, 1715, 1680. Mass spectrum, m/e (%): 602 (M^+; 0.5), 587 (0.5), 584 (0.5), 574 (0.5), 566 (2), 556 (3), 551 (1), 538 (30), 524 (13), 523 (23), 520 (5), 506 (83), 496 (11), 491 (16), 480 (33), 448 (43), 433 (30), 417 (11), 411 (5), 403 (3), 400 (43), 399 (87), 393 (12), 385 (5), 367 (4), 341 (39), 340 (8), 290 (7), 289 (8), 183 (67), 140 (57), 139 (100), 43 (90). The acetonide (III) is a very thermolabile compound. For analysis, the substance was dried in vacuum at 25-27°C. When an attempt was made to dry it at 80°C, it readily hydrolyzed.

SUMMARY

The leaves of *Ajuga turkestanica* (Rgl.) Briq. have yielded a new ecdysone — 22-acetylcyasterone. The mass spectrum of this new ecdysone and the features of it due to the presence of the acetoxy group at C-22 have been discussed.

LITERATURE CITED

1. B. Z. Usmanov, M. B. Gorovits, and N. K. Abubakirov, Khim. Prirodn. Soedin., 535 (1971); 125 (1973); 466 (1975).
2. B. Z. Usmanov, Z. Saatov, and N. K. Abubakirov, Khim. Prirodn. Soedin., 422, 710 (1977).
3. T. Takemoto, Y. Hikino, K. Nomoto, and H. Hikino, Tetrahedron Lett., 3191 (1967).
4. H. Hikino, Y. Hikino, K. Nomoto, and T. Takemoto, Tetrahedron, 24, 4895 (1968).
5. G. Spiteller, Z. Anal. Chem., 197, 1 (1963).
6. Ya. V. Rashkes, M. B. Gorovits, G. K. Makarichev, and N. K. Abubakirov, Khim. Prirodn. Soedin., 747 (1971).