A STUDY OF THE HERB Aerva lanata.

III. ALKALOIDS

G. G. Zapesochnaya, L. N. Pervykh, and V. A. Kurkin

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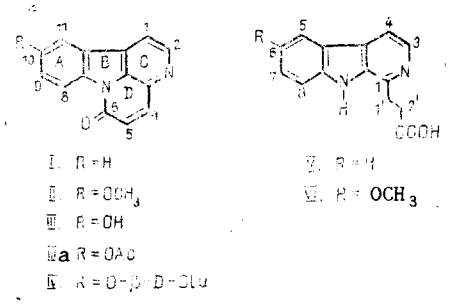
Four new alkaloids – aervine (10-hydroxycanthin-6-one), methylaervine (10-methoxycanthin-6-one), aervoside (10-β-D-glucopyranosyloxycanthin-6-one), and aervolanine (3-(6-methyoxy-β-carbolin-1-yl)propionic acid), and also the known alkaloids canthin-6-one and 3-(β-carbolin-1-yl)propionic acid have been isolated from the herb Aerva lanata Juss. Their structures have been established on the basis of chemical and spectral characteristics.

In the course of a study of the chemical composition of the herb Aerva lanata Juss. (Amaranthaceae), which possesses diuretic properties [1], p-coumaroyl glycosides of flavenoids [2], and feruloylamides of tyramine and of homovanillylamine and also other phenolic compounds [3] have previously been isolated

Extraction of Aerva lanata (85% ethanol), the distribution of the extractive substances between chloroform, ethyl acetate, and water, the chromatography of the evaporated extracts on silica gel, and repeated rechromatography with a sequence of sorbents (silica gel, polyamide, Sephadex LH-20) and of eluent mixtures the components of which were hexane, chloroform, and methanol, led to the isolation of alkaloids of the canthinone and β-carboline types (I-VI).



I R = H
II R = OCH_3
III R = OH
IIIa R = OAc
IV R = O-β-D-Glu

V R = H
VI R = OCH_3

The structures of the alkaloids isolated were studied by the chemical transformations shown in part in Scheme 1 and also by a comparison of the physicochemical constants and characteristics of these groups of compounds (UV, IR, NMR, and mass spectra) with those given in the literature [4-9].

The PMR spectrum of all the canthin-6-one alkaloids isolated (I-IV) showed the absence of substituents in rings C and D, as witnessed by characteristic doublets of the H-4 and H-5 protons with the constant J = 10 Hz, and also by the doublets of a pair of vicinal protons (H-1 and H-2) (Table 1) similar with respect to chemical shifts and constants (J = 5 Hz) to the analogous pair in positions 3 and 4 of the β-carbolines (V) and (VI).

Unsubstituted canthin-6-one (I) is widely distributed in plants, and all the results that we obtained coincided with those given in the literature [4]. Thus, in addition to the signals of the protons of rings C and D (H-1, -2, -3, and -4) that have been mentioned, its PMR spectrum also contained the signals of four aromatic protons that are characteristic for a canthinone with an unsubstituted ring A. The molecular ion (M^+ 220) and the fragmentation in the mass spectrum also corresponded to structure (I).

Each of the other canthin-6-ones (II-IV) contained a single substituent in Ring A. Their NMR and mass spectra showed that these substituents were: in (II), a methoxy group; in (III), a hydroxy group; and in (IV), a glucose residue. The nature of the splitting of the signals

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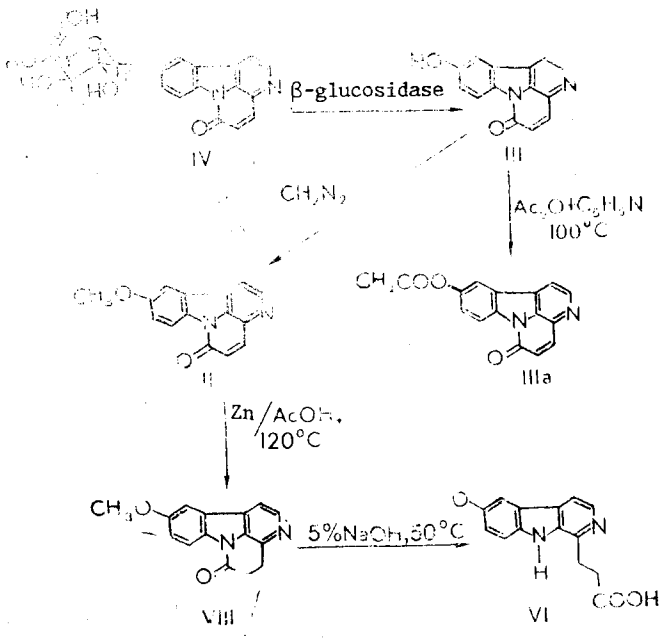
TABLE 1. ^{1}H NMR Results for the Canthin-6-ones Isolated (II-IV)

Proton	Chemical shifts (δ, ppm) of the signals of the protons of the compounds in the given solvents					
	II $CDCl_3$	II C_5D_5N	III DMSO-d_6	IIIa $CDCl_3$	IV C_5D_5N	IV DMSO-d_6
H-1 d, 5 Hz	7,90	7,93	8,28	7,90	7,92	8,22
H-2 d, 5 Hz	8,80	8,82	8,82	8,80	8,83	8,78
H-4 d, 10 Hz	8,00	8,02	8,12	8,01	8,04	8,08
H-5 d, 10 Hz	6,97	6,98	6,97	6,97	7,00	6,98
H-8 d, 8,5 Hz	8,50	8,72	8,34	8,74	8,71	8,34
H-9 dd, 8,5 and 2 Hz	7,22	7,48	7,20	7,41	7,69	7,45
H-11 d, 2 Hz	7,52	7,88	7,70	7,87	8,25	7,95
CH_3O — s	3,98		10,0			
OH — s				2,41		
CH_3CO — s					5,79	5,09
H—1' of glucose — d					7,3 Hz	8,0Hz

of the three aromatic protons of ring A in the PMR spectra (Table 1) showed that the substituent in each of compounds (II)-(IV) could be attached at position 9 or 10 of the canthine system. The choice in favor of 10-substituted canthin-6-ones was made on the basis of a comparison with literature information [4-9] with the following considerations. In compound (II), the methoxy group must be located at C-10, since the H-8 signal is located in the weak field (δ 8.50) and its ortho interaction (J = 8.5 Hz) excludes the presence of a substituent at C-9. The analogous characteristics of the H-8 signal in each of compounds (III), (IIIa), and (IV) (Table 1) also indicate the location of the corresponding substituent at C-10. It must be mentioned that the assignment of the signals in the ^{1}H NMR spectra of compound (IV) was made on the basis of the results of double resonance.

An additional confirmation of the conclusions made concerning the structures of compound (II-IV) was given by the scheme of their chemical transformations. Thus, compound (IV), which has been called aervoside, was readily hydrolyzed by β-glucosidase with the formation of aervine (III), which, on acetylation (the process taking place only on heating) formed a monoacetate (IIIa) and on methylation with diazomethane was converted into methylaervine (II). All the constants and spectral characteristics of the native compounds and those obtained in the course of the transformations coincided.



Scheme of the chemical transformations of compounds (II-IV).

We are the first to have isolated compound (IV) (10-β-D-glucopyranosyloxycanthin-6-one). The following explanations must be given relative to compounds (II) and (III). We regard

TABLE 2. Constants and Chemical Shifts of the Aromatic Protons of 1,2,4-Substituted Ring A of Some Canthin-6-ones

Compound	mp, °C	Signals of ring A in the ^{1}H NMR spectrum (δ, ppm)		
		d 8,5 Hz	d 2 Hz	dd 8,5 and 2 Hz
Methylaervine (II)	194—196	8,50	7,52	7,22
10-Methoxycanthin-6-one[5]	175—178	7,74	7,97	6,91
9-Methoxycanthin-6-one [6]	175—176	7,85	8,13	7,01
9-Methoxycanthine-6-one[7]	178—180	7,96	8,22	7,08
Aervine (III)	310—313 (decom.)	8,72	7,88	7,48*
		8,34	7,70	7,20**
10-Hydroxycanthin-6-one[5]	288—293 (decom.)	8,14	7,99	6,99**
Aervine acetate (IIIa)	186—188	8,74	7,87	7,41
10-Acetoxycanthin-6-one[5]	240	8,08	8,42	7,27
Aervoside (IV)	215—218	8,71	8,25	7,69*
		8,34	7,95	7,45**

*Solvent — C_5D_5N.

**Solvent — DMSO-d_6. The other spectra were taken in $CDCl_3$.

them as new compounds although analogous structures have been described in a single paper by Arisawa et al [5]. However, our results show the necessity for a revision of the statements in [5]. The 10-methoxycanthin-6-one described in the present paper agrees most closely with respect to its melting point and the PMR siganls of ring A (Table 2) to the structure of 9-methoxycanthin-6-one [6, 7] isolated from the same plant (Simaba multiflora [7]). Methylaervine (II) differs substantially from 9-methoxycanthin-6-one and the substance described in [5] both in melting point and with respect to the chemical shifts of the signals of ring A (Table 2).

Similar noncorrespondences of the ^{1}H NMR characteristics and the melting points (Table 2) were also observed for aervine (10-hydroxycanthin-6-one (III)) and its acetate (IIIa) on the one hand and the substances of analogous structure described by Arisawa et al. [5] which, in our opinion, must be assigned to the corresponding 9-isomers.

The β-carboline compounds isolated ((V) and (VI)) were closely related to the compounds (I) and (II) of canthin-6-one nature. Thus, the hydrogenation product (VII) obtained from the canthinone (I) and the 3-β-carbolin-1-yl propionic acid (V) obtained on its subsequent saponification coincided completely with their descriptions in the literature [8].

The ^{1}H NMR spectra of the new compound (VI) (aervolanine) differed from that of 3-(β-carboline-1-yl) propionic acid (V) [8], which we had also isolated from the plant, only by the signals of ring A (δ 7.78, d 2 Hz, H-5; δ 7.23, dd 9 and 2 Hz, H-7; and δ 7.55, d 9 Hz, H-8) and by the presence of a methoxy group (δ 3.92) which was assigned to C-6.

The correctness of the proposed structure of aervolanine (3-(6-methoxy-β-carbolin-1-yl) propionic acid) was confirmed by the formation of this compound from substance (II) by reduction with Zn/AcOH and hydrolysis of the resulting lactam (VIII) on heating with 5% NaOH (see scheme).

It is interesting that the alkaloids isolated had a characteristic bright blue or yellow-green fluorescence, which can be used in chemotaxonomic investigations and, in particular, in connection with the search for them in plant materials.

EXPERIMENTAL

Spectral characteristics were obtained on the following instruments: Varian HA-100D, Bruker-250, and Bruker-500 (^{1}H NMR: δ scale, 0—TMS); Varian CH-8 at 70 eV (mass spectra); Specord M40 and Hitachi EPS-3T in ethanol and with the addition of a 0.1 M solution of NaOMe and of 10% HCl (UV); and UR-20, paraffin oil (IR). Elementary analyses were determined on a Hewlett-Packard 185B automatic CHN analyzer. TLC monitoring was conducted on Silufol UV 254 plates in the solvent systems chloroform—methanol—water (26:14:3) and chloroform—methanol (6:1), the chromatograms being viewed in UV light at wavelengths of 254 and 360 nm and being visualized with Ehrlich reagent. The results of elementary analysis corresponded to the calculated values.

Isolation of the Alkaloids. The comminuted air-dry herb Aerva lanata gathered in Transcaucasia [1] (2 kg) was extracted with 85% ethanol (1:10) three times, the extract was evaporated in vacuum to an aqueous residue having a volume of 0.5 liter, and this was extracted with chloroform (5 × 0.2 liter) and with ethyl acetate (5 × 0.2 liter). The aqueous residue and the evaporated extracts were dried with silica gel L 40/100, the aqueous residue yielding 200 g of extractive substances and the chloroform and ethyl acetate extracts 50 g each of extractive substances. Many-times repeated chromatography of the dried extracts on silica gel, polyamide, or Sephadex LH-20 (using the eluent mixtures hexane–chloroform and chloroform–methanol in various ratios) led to compounds (I-VI).

Canthin-6-one (I). Slightly yellowing acicular crystals with a light blue fluorescence at 360 nm, composition $C_{14}H_{18}N_2O_2$, mp 155-156°C (from ethanol). Maxima in the UV spectra (nm, log ε): EtOH – 251(4.10), 259(4.12), 268(4.07), 300(3.92), 347(3.94), 362(4.15), 380 (4.13); NaOMe and HCl – no changes. IR (cm^{-1}): 1670, 1630 (C=O, conjugated); 1600 (arom.). PMR spectrum in $CDCl_3$ (δ, ppm): 7.88 (d, 5 Hz, H-1), 8.78 (d, 5 Hz, H-2), 7.96 (d, 10 Hz, H-4), 6.95 (d, 10 Hz, H-5), 8.62 (d, 8.5 Hz, H-8), 7.66 (t, 8.5 Hz, H-9), 7.48 (t, 8.5 Hz, H-10), 8.06 (d, 8.5 Hz, H-11).

Mass-spectrum (m/z, %): M^+ 220(100), 192(87), 165(37), 164(43), 139(37), 110(22).

10-Methoxycanthin-6-one (Methylaervine) (II). Bright yellow crystals with a bright yellow-green fluorescence at 360 nm; composition $C_{15}H_{10}N_2O_2$, mp 194-196°C (from MeOH or chloroform–ethanol). Yield 0.02% on the weight of the air-dry raw material. Maxima in the UV spectra (nm, log ε): EtOH 269 sh. (4.31), 277(4.41), 297 sh. (3.93), 308 sh. (3.90), 355 sh. (4.01), 376(4.09); EtOH + NaOMe initial spectrum: EtOH + HCl 269 sh., 278(4.30), 310 sh. (3.92), 323 sh. (3.83), 361(4.08), 380(4.08).

IR (cm^{-1}): 1670, 1635 (C=O, conjugated); 1610, 1575 (arom.). For the PMR spectrum, see Table 1. Mass spectrum (m/z, %): M^+ 250(100), 235(88), 207(21), 179(15), 153(8), 125(9).

10-Hydroxycanthin-6-one (Aervine) (III). Yellow crystals with the composition $C_{14}H_8N_2O_2$, mp 310-313°C (decomp) (from MeOH or chloroform–ethanol). Maxima in the UV spectra (nm, log ε): EtOH 271 sh. (4.22), 279(4.30), 297 sh. (3.87), 308 sh. (3.82), 358 sh. (3.96), 375(3.97): EtOH + NaOMe 299(4.31), 358(3.96), 376(3.85), 460(3.51); EtOH + HCl 280(4.24), 310 sh. (3.93), 323(3.89), 361(3.98), 377(3.95).

IR (cm^{-1}): 1670. For the PMR spectrum, see Table 1. Mass spectrum (m/z, %): M^+ 236(100), 208(66), 179(20), 153(7), 127(4), 118(12).

Acetylation of 10-Hydroxycanthin-6-one (III). 10-Hydroxycanthin-6-one (III) (20 mg) was heated with a 1:1 mixture of pyridine and acetic anhydride (1 ml) on the boiling water bath for 1 h. The usual treatment with water and recrystallization from ethanol led to the monoacetate (IIIa) (15 mg) in the form of colorless acicular crystals with a light blue fluorescence at 360 nm, mp 186-188°C; composition $C_{16}H_{10}N_2O_3$, M^+ 278. Maxima in the UV spectra (nm, log ε): EtOH 252(3.94), 262(3.97), 271(4.00), 292(3.70), 301 sh. (3.69), 366(3.88), 385(3.87); EtOH + NaOMe 300(4.12), 361(3.72), 380(3.67); EtOH + HCl 262(3.99), 272(4.02), 305(3.77), 366(3.93), 385(3.92). For the PMR spectrum, see Table 1.

Methylation of 10-Hydroxycanthin-6-one (III). A methanolic solution of compound (III) (15 mg) was treated with an ethereal solution of diazomethane at room temperature for 48 h (portions of a fresh solution of CH_2N_2 were added several times). After the solvents had been driven off, the residue was chromatographed on silica gel. The desired product was desorbed with chloroform–hexane (5:5→6:4) and, after the evaporation of the eluates, was recrystallized from MeOH. Yellow needles (10 mg) with mp 193-195°C, M^+ 250 were obtained. The substance was shown to be identical with compound (II) by a direct comparison.

Aervoside (10-β-D-Glucopyranosyloxycanthin-6-one) (IV). Light yellow crystals with the composition $C_{20}H_{18}N_2O_7$, mp 215-218°C (from MeOH or EtOH). Maxima in the UV spectra (nm, log ε): EtOH 265(4.08), 273(4.15), 360(3.86), 377(3.82); with additions of NaOMe and HCl the spectrum did not change. IR (cm^{-1}): 3350, 1670, 1660, 1640, 1610, 1575. PMR spectrum (the signals of the aromatic protons are given in Table 1) in C_5D_5N (ppm) (500 MHz): 5.79 (D, 7.3 Hz, H-1'), 4.66 (dd, 12 and 2 Hz, H-6'), 4.45 (m, 3H 4-glucose), 4.37 (m, H-5'), 4.25 (m, 12 Hz, H-6').

Enzymatic Hydrolysis of Aervoside (IV). A solution of 5 mg of aervoside (IV) in 1 ml of ethanol was diluted with 4 ml of water, and the resulting suspension was heated with

a solution of 1 mg of β-glucosidase (Serva) in 0.5 ml of water at 38-40°C. Hydrolysis was complete in 1 h. Glucose was detected in the hydrolysate. An ethereal extract of the aglycon, after evaporation, was recrystallized from ethanol to give yellow crystals with decomp. 310°C, M^+ 236. The substance was shown to be identical with compound (III) by a direct comparison.

3-(β-Carbolin-1-yl)propionic Acid (V). Slightly yellowish acicular crystals with a light blue fluorescence at 254 and 360 nm; composition $C_{14}H_{12}N_2O_2$, M^+ 240, mp 214-215°C (from acetone–water (1:1)). Maxima in the UV spectra (nm, log ε): EtOH 236(4.92), 280 sh. (4.45), 290(4.50), 300 infl. (4.40), 336(4.36), 350(4.36); EtOH + NaOMe – initial; EtOH + HCl 251(4.97), 303(4.80), 370(4.45).

PMR spectrum in C_5D_5N (ppm): 8.52 (d, 5 Hz, H-3), 7.92 (d, 5 Hz, H-4), 8.20 (d, 9 Hz, H-5), 7.2-7.6 (m, H-6,7,8), 3.80 (t, 7.5 Hz, 2H-1'), 3.34 (t, 7.5 Hz, 2H-2'). Mass spectrum (m/z, %): M^+ 240(80), (M-18) 222(100), 195(45), 194(60), 193(50), 181(30), 168(47), 167(35), 140(49).

Synthesis of (V). Canthin-6-one (I) (50 mg) in acetic acid (2 ml) was heated at the boil with granulated zinc for 30 min. The solution was diluted with water (2 ml) and was neutralized by the addition of Na_2CO_3. The precipitate that deposited was washed with water and was recrystallized from hexane to give 4,5-dihydrocanthin-6-one (VII) (M^+ 222, mp 130°C, bright yellow-green fluorescence in UV light at 360 nm) (40 mg). The compound (VII) was dissolved in 1.5 ml of methanol and the solution was heated at 50°C with 2 ml of 5% NaOH for 30 min. The resulting solution was acidified with 10% HCl and the needles that deposited were washed with water and recrystallized from water–acetone (1:1) to give colorless acicular crystals of compound (V) (30 mg) with the composition $C_{14}H_{12}N_2O_2$ (M^+ 240), mp 215-216°C.

Aervolanine (3-(6-Methoxy-β-carbolin-1-yl)propionic Acid) (VI). Yellow acicular crystals with a bright yellow-green fluorescence at 360 nm; composition $C_{15}H_{14}N_2O_3$, mp 194-196°C (from ethanol). Yield 0.01% on the weight of the air-dry raw material. Maxima in the UV spectra (nm, log ε): EtOH 234(4.71), 250 sh. (4.57), 292 sh. (4.32), 299(4.47), 310 sh. (4.11), 358(3.63), 375 sh. (3.62); EtOH + NaOMe 295(4.41), 301(4.56), 357(4.06), 375(4.06); EtOH + HCl 238(4.59), 267(4.57), 314(4.53), 410(3.89).

IR (cm^{-1}): 1660, 1640, 1620, 1580. PMR spectrum in DMSO–d_6 (ppm): 11.15 (s, NH), 8.25 (d, 5 Hz, H-3), 7.95 (d, 5 Hz, H-4), 7.78 (d, 2 Hz, H-5), 7.23 (dd, 9 and 2 Hz, H-7), 7.55 (d, 9 Hz, H-8), 3.92 (s, CH_3O), 3.37 (m, 7.5 Hz, 2H-1'), 2.90 (m, 7.5 Hz, 2H-2'); C_5D_5N (ppm): 8.50 (H-3), 7.93 (H-4), 7.83 (H-5), 7.28 (H-7), 7.55 (H-8), 3.85 (CH_3O), 3.75 (2H-1'), 3.33 (2H-2').

Mass spectrum (m/z, %): M^+ 270(58), 252(100), 240(24), 237(26), 225(71), 224(78), 209 (100), 195(82), 181(38), 168(22), 153(13), 140(31), 127(15), 126(18).

Synthesis of (VI). 10-Methoxycanthin-6-one (II) (100 mg, in 3 ml of AcOH) was treated with granulated zinc in a similar way to compound (I). This yielded 80 mg of 4,5-dihydromethylaervine (VIII) (M^+ 252; mp 153-154°C from MeOH; bright yellow-green fluorescence in UV light at 360 nm). The compound (VIII) was dissolved in 2 ml of MeOH and the solution was treated with 5% NaOH, as for compound (VII). This gave 63 mg of yellow acicular crystals (compound (VI)) with mp 185-188°C; after additional recrystallization from ethanol–chloroform, mp 193-195°C; M^+ 270, $C_{15}H_{14}N_2O_3$.

LITERATURE CITED

1. M. Udupihille and M. T. M. Jiffry, Indian J. Physiol. Pharmacol, 30, No. 1, 91 (1986).
2. A. M. Zadorozhnyi, G. G. Zapesochnaya, L. N. Pervykh, et al., Khim.-Farm. Zh., 20, No. 7, 855 (1986).
3. G. G. Zapesochnaya, V. A. Kurkin, and L. N. Pervykh, Khim. Prir. Soedin., No. 5, 694 (1990).
4. T. Ohmoto, R. Tanaka, and T. Nikaido, Chem. Pharm. Bull, 24, No. 7, 1532 (1976).
5. M. Arisawa, A. D. Kinghorn, G. A. Cordell, et al., J. Nat. Prod., 46, No. 2, 222 (1983).
6. A. M. Giesbrecht, H. E. Gottlieb, O. R. Gottlieb, et al., Phytochemistry, 19, No. 2, 313 (1980).
7. J. Polonsky, J. Gallas, J. Varenne, et al., Tetrahedron Lett., 23, No. 8, 869 (1982).
8. T. Ohmoto and K. Koike, Chem. Pharm. Bull., 32, No. 1, 170 (1984).
9. J. R. F. Allen and B. R. Holmstedt, Phytochemistry, 19, No. 8, 1573 (1980).