ISOEVONINE, A NOVEL ALKALOID FROM *Euonymus Europaea* L. CONTAINING WILFORDIC ACID

Ľ.DÚBRAVKOVÁ[a], L.DOLEJŠ[b] and J.TOMKO[a]

[a] *Institute of Chemistry, Slovak Academy of Sciences, 809 33 Bratislava and*
[b] *Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague*

Received October 23rd, 1972

A new alkaloid, isoevonine, was isolated from the seeds of *Euonymus europaea* L. in addition to the major alkaloid evonine. Isoevonine possesses the same molecular formula $C_{36}H_{43}NO_{17}$ as evonine, but instead of evoninic [2-(1-methyl-2-carboxypropyl)nicotinic] acid it contains the isomeric wilfordic [2-(3-carboxybutyl)nicotinic] acid. The sesquiterpenic moiety of the molecule is identical in both alkaloids.

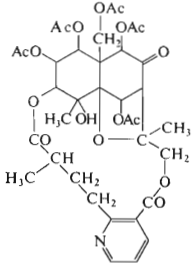
Recently, alkaloids of the genus *Euonymus* were studied by several authors. Wada and associates[1-3] were the first who reported the structure and stereochemistry of evonine, the major alkaloid isolated from the above-mentioned plants. Also papers by other authors[4,5] dealing with the same topic were published. The structure and stereochemistry of evonine has independently been proved by the X-ray diffraction analysis method[6]. The structures of further alkaloids, isolated from *Euonymus* plants, were determined as well[3,4,7-9].

Seeds of *Euonymus europaea* L. contain several alkaloids, as was shown by thin-layer chromatography. Some of the alkaloids seemed to be novel and therefore, we investigated them in more detail. After separation of the major alkaloid, mother liquors were chromatographed and gave two minor bases. The first of them was shown to be identical with evonoline[4] the second, less polar for which we propose the name isoevonine, is a new one.

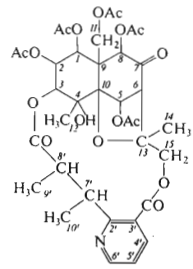
Isoevonine thus isolated exhibits, likewise evonine, a molecular ion peak at *m/e* 761·2501 (calculated 761·2531) corresponding to the molecular formula $C_{36}H_{43}$. NO_{17} Intense ionic species at *m/e* 206 ($C_{11}H_{12}NO_3$) were encountered with both alkaloids. According to arguments, adduced later on, we formulate the structure of the ion at *m/e* 206 of isoevonine as *a*); the species at *m/e* 178 could be the decarbonylation product of *a*). Differences between isoevonine and evonine were found in some physicochemical constants and spectral data.

As seen from the PMR spectra of isoevonine (*I*) and evonine (*II*) (Table I), the position of the proton signals in the sesquiterpenic moiety of the molecule (H-1 to H-15) is the same except for protons H-3 and OH-4, which are downfield shifted. The paramagnetic shift can indicate a various stereochemical arrangement of substituents in alkaloids concerned. A substantial difference between the PMR spectra of both

alkaloids is seen in the position of protons in the acid moiety of the molecule. Isoevonine reveals only one doublet at 1·21 p.p.m. (3 H, d, $J = 7{\cdot}0$ Hz) characteristic of a sec-methyl group in the α-position to the ester grouping, whereas evonine does

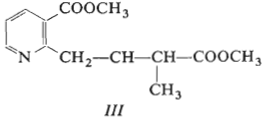


I

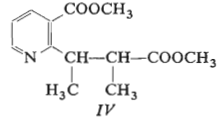


II

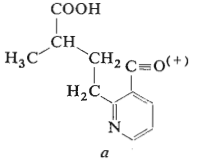
two doublets of a sec-methyl groups at 1·21 p.p.m. (3 H, d, $J = 7{\cdot}0$ Hz) and 1·41 p.p.m. (3 H, d, $J = 7{\cdot}0$ Hz). Further signals in the PMR spectrum of isoevonine were ascribed to protons of methylene groups of wilfordic acid (two multiplets centered at about 2·35 and 2·85 p.p.m.). The proton, which is in the neighbourhood of the sec-methyl group in isoevonine resonates at about 2·82 p.p.m. (m), whilst that of evonine at 2·59 p.p.m. (H, *q*, $J = 7{\cdot}0$ Hz). Also the ORD curves of *I* and *II* are dif-



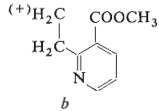
III



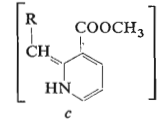
IV



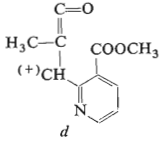
a



b



c



d

ferent. Isoevonine displays a negative Cotton effect $[\Phi]_{237} - 9\,630°$, evonine a positive one $[\Phi]_{232} + 9\,550°$. Further course of the ORD curves evidences that the stereochemical arrangement of the molecule in the vicinity of the carbonyl group is identical in both alkaloids.

Upon methanolysis isoevonine afforded dimethyl wilfordate (*III*), evonine dimethyl evoninate[8] (*IV*). Mass spectra of both esters exhibit the same molecular ion peaks at *m/e* 251, corresponding to $C_{13}H_{17}NO_4$, nonetheless the fragmentation pattern of those substances was found to be considerably different. The spectrum of dimethyl wilfordate (*III*) displays characteristic fragment ions (*b*) at *m/e* 164 (100%, $C_9H_{10}NO_2$) analogous to those formed from 2-ethylpyridines by γ-cleavage, and ions (*c*) (R = H) at *m/e* 151 (79·4%, $C_8H_9NO_2$, McLafferty rearrangement)[10].

TABLE I

Values of the PMR Spectra

PMR spectra (on δ scale in p.p.m.) were measured in $CDCl_3$ at 80 MHz, tetramethylsilane being the internal reference substance. Multiplicities and coupling constants are in parentheses. Protons are assigned according to[5].

Proton	Isoevonine (*I*)	Evonine (*II*)
H-1	5·70 (d 3·5)	5·67 (d 3·5)
H-2	5·15 (t 3·2)	5·27 (t 3·2)
H-3	4·97 (d 3·0)	4·76 (d 3·0)
H-5	6·72 (d 1·0)	6·76 (d 1·0)
H-6	3·02 (d 1·0)	3·04 (d 1·0)
H-8	5·53 (s)	5·55 (s)
H-11	4·47, 4·85 (AB q 13·0)	4·53, 4·81 (AB q 13·0)
H-12	1·61 (s)	1·61 (s)
H-14	1·55 (s)	1·61 (s)
H-15	3·79 (d 12·0), 5·81 (d 12·0)	3·71 (d 12·0), 6·02 (d 12·0)
OH-4	5·12 ($J_{12,OH} \neq 0$)	4·63 ($J_{12,OH} \neq 0$)
H-4′	8·28 (dd 8·0, 2·0)	8·09 (dd 8·0, 2·0)
H-5′	7·26 (dd 8·0, 5·0)	7·25 (dd 8·0, 5·0)
H-6′	8·73 (dd 5·0, 2·0)	8·69 (dd 5·0, 2·0)
Acetyls	1·90, 2·02, 2·10, 2·13, 2·20	1·89, 2·05, 2·10, 2·15, 2·23

The mass spectrum of dimethyl evoninate showed species (*d*) at *m/e* 204 (100%) and (*c*) ($R = CH_3$) at *m/e* 165 (84·7%, McLafferty rearrangement). In the PMR spectrum of dimethyl wilfordate signals due to one sec-methyl group and a proton of the methine group appeared at 1·23 (*d*, $J = 7·0$ Hz) and 2·67 p.p.m. (m), respectively. The signal at 3·16 p.p.m. (2 H, t, $J = 4·0$ Hz) was ascribed to protons of the methylene group in the neighbourhood of the pyridine ring, that at 2·08 (m) to the further methylene group.

As it follows, isoevonine (*I*) differs from evonine (*II*) by the nature of esterifying and is the first alkaloid isolated from *Euonymus europaea* L. in which the sesquiterpenic moiety of the molecule is bound to wilfordic acid[11].

EXPERIMENTAL

Melting points (uncorrected) were measured on a Koffler micro hot-stage. Mass spectra were recorded on an AEI MS spectrograph, optical rotation on a Perkin-Elmer polarimeter, IR spectra on a UR-10 spectrophotometer, UV spectra and ORD curves on a UV/ORD Jasco 5 spectropolarimeter. The PMR spectra were measured on a Tesla 487 apparatus. Silica gel (Merck 0·05—0·2 mm) was employed for column chromatography, silica gel G according to Stahl (Meck) for thin layer chromatography. Coated plates were activated at 140—150°C for 3 h. Chromatograms were detected with Dragendorff's reagent.

Isolation of Alkaloids

The arils of fruits of *Euonymus europaea* L. collected near Bratislava in the autumn, were peeled off. The seeds (12·6 kg) were airdried, ground and extracted with a 10-fold amount of heptane and allowed to stand for several hours. After the suspension had sedimented the heptane solution was decanted and extracted with 2% HCl. The acid layer was basified with sodium carbonate and alkaloids were recovered with ether. This procedure was repeated 10 times. The combined ethereal extracts evaporated to dryness yielded 21·8 g of bases (0·17% on the weight of seeds employed).

Evonine: Diethyl ether was gradually added to a solution of the alkaloid mixture (13·7 g) in benzene (400 ml) till first crystals separate. The mixture was allowed to stand at room temperature for several hours and then the crystals (7·1 g), which consisted of evonine and a small amount of minor alkaloids, were filtered off. Evonine ($C_{36}H_{43}NO_{17}$) obtained by chromatography on silica gel column with ether as eluent had m.p. 183—188°C (ethanol), $[\alpha]_D$ +21·1° (*c* 1·46, ethanol), M^+ 761·2492 (6·8%) (calculated 761·2531), other species at *m/e* 733 (4·5), 719 (3·6), 718 (3·5) 703 (2·6), 702 (4·5), 659 (2·1), 658 (4·0), 642 (2·6), 528 (2·7), 262 (3·7), 222 (3·1), 220 (8·2), 219 (4·2), 218 (5·1), 208 (3·2), 207 (9·5), 206 (58·5), 204 (7·5), 190 (5), 179 (10), 178 (72), 176 (5), 162 (9), 161 (20), 160 (23), 159 (7), 152 (8), 151 (13), 150 (17), 146 (10), 134 (23), 133 (25), 132 (20), 118 (7), 117 (6), 108 (10), 107 (95), 106 (25), 105 (7), 93 (7), 83 (5), 55 (10), 43 (100), 41 (5). IR Spectrum (tetrachloromethane): 1 750 (sh), 1 780, 3 490 cm^{-1}; UV spectrum (ethanol): $[\lambda]_{max}$ 222, 265 nm ($\log \varepsilon$ 3·90, 3·56); ORD (ethanol): $[\Phi]_{310}$ +7 930° (max), $[\Phi]_{290}$ −3 010° (sh), $[\Phi]_{272}$ −15 880° (min), $[\Phi]_{250}$ −3 290° (sh), $[\Phi]_{232}$ +9 550° (max), $[\Phi]_{215}$ −2 476° (min). R_F 0·78 (chloroform–methanol 98 : 2) and 0·46 (diethyl ether).

Evonoline: Mother liquors after separation of evonine (2·67 g), chromatographed on a silica gel (50 g), column, afforded 40 ethereal fractions 15 ml each. Fractions 1—4 gave a mixture of three alkaloids (381·0 mg): evonoline, evonine and an unidentified base of R_F 0·52. Evonoline was separated by repeated chromatography on a silica gel column, ether and benzene–ethanol (98 : 2) being the eluents. Amorphous ($C_{36}H_{43}NO_{16}$, 127·6 mg), $[\alpha]_D$ +6·2° (*c* 0·83, ethanol), M^+ 745·2583 (3·1) (calculated 745·2582), further species at *m/e* 701 (3·4), 686 (5·4), 642 (4·4), 423 (2·4), 206 (23), 178 (82), 161 (20), 160 (18), 107 (82), 105 (95). IR spectrum (tetrachloromethane): 1 760 (sh), 1 780 cm^{-1}; UV spectrum (ethanol): $[\lambda]_{max}$ 222, 265 nm ($\log \varepsilon$ 3·90, 3·56); ORD (ethanol): $[\Phi]_{310}$ +13 660° (max), $[\Phi]_{290}$ 0°, $[\Phi]_{266}$ −16 720° (min), $[\Phi]_{246}$ −7 390° (max) $[\Phi]_{237}$ −11 450° (sh), $[\Phi]_{212}$ −13 700° (min). R_F 0·87 (chloroform–methanol 98 : 2), 0·60 (diethyl ether).

Isoevonine: Further fractions (7—12) of the above-mentioned chromatography furnished evonine (740 mg). Fraction 15—20 afforded a mixture of less polar, hitherto unidentified alkaloids

(549·2 mg). The last fractions (21—40) gave a mixture of alkaloids (297·8 mg) from which amorphous isoevonine (110·0 mg) was obtained by rechromatography on silica gel column. Isoevonine ($C_{36}H_{43}NO_{17}$), amorph., $[\alpha]_D$ +30·5 (*c* 0·90 ethanol) M^+ 761·2501 (11·1%) (calculated 761·2531), other peaks at *m/e* 717 (3·2), 703 (7·4), 702(8·5), 659 (4·0), 658 (7·2), 645 (4·8), 644 (13·3), 262 (3·5), 234(3·2), 222 (4·8), 219 (2·9), 218 (7·2), 207 (6·9), 206 (42), 178 (58), 161 (18), 160 (16), 150 (11), 147 (14), 134 (13), 133 (13), 132 (23), 129 (27), 124 (13), 107 (18), 106 (20), 93 (98), 83 (10), 71 (13), 70 (11), 69 (10), 57 (24), 55 (15), 43 (100), 41 (17). IR Spectrum (tetrachloromethane): 1750 (sh), 1780, 3470 cm^{-1}; UV spectrum (ethanol): $[\lambda]_{max}$ 224, 271 nm (log ε 4·04, 3·59): ORD (ethanol): $[\Phi]_{310}$ +7850° (max), $[\Phi]_{290}$ 0°, $[\Phi]_{267}$ −12610° (min), $[\Phi]_{249}$ −5690° (max), $[\Phi]_{237}$ −9630° (min), $[\Phi]_{221}$ +2850° (max).

Methanolysis of Isoevonine

Isoevonine (83·4 mg) in benzene (3 ml) was treated with 0·16M-$NaOCH_3$ (3·2 ml) in methanol at 15°C for 24 h under a nitrogen atmosphere. The solvent was recovered under diminished pressure and the residue was extracted with benzene (3 × 20 ml). Dimethyl 2-(3-carboxybutyl)nicotinate (dimethyl wilfordate) thus obtained (19·8 mg) was purified on a silica gel column. Yield 13·5 mg (49%), oil, $[\alpha]_D$ +17 ± 2° (1·76; ethanol). For $C_{13}H_{17}NO_4$ M^+ 251·1661 (0·9) Calc. 251·157 other peaks at *m/e* 236 (5·5), 220 (22), 204 (6), 193 (8), 192 (68), 176 (4), 165 (12), 164 (100), 160 (15), 158 (4), 152 (7), 151 (79), 150 (6), 145 (5), 144 (8), 136 (12), 133 (6), 132 (40), 131 (5), 130 (14), 120 (5), 118 (7), 117 (18), 116 (5), 104 (16), 94 (7), 93 (71), 92 (14), 91 (12), 79 (13), 78 (11), 65 (13), 64 (7), 63 (7), 59 (7), 52 (5), 51 (9), 41 (7), 39 (10). R_F 0·38 (chloroform–methanol 99 : 1).

Methanolysis of Evonine

Evonine (76·1 mg) was saponified in the same manner as isoevonine. Yield 8·8 mg (35%) of dimethyl 2-(1-methyl-2-carboxypropyl)nicotinate (dimethyl evoninate), oil $[\alpha]_D$ −55 ± 2° (*c* 0·98, ethanol), M 251 (9), 237 (8), 236 (54), 220 (31), 205 (13), 204 (100), 193 (8), 192 (59), 176 (16), 166 (9), 165 (85), 164 (34), 162 (8), 161 (12), 160 (53), 158 (8), 151 (28), 150 (67), 148 (5), 146 (7), 145 (13), 144 (19), 138 (7), 134 (5), 133 (11), 132 (76), 131 (10), 80 (6), 79 (47), 78 (35), 77 (31), 76 (7), 67 (4), 66 (5), 65 (15), 64 (5), 63 (10), 59 (15), 56 (4), 55 (10), 53 (11), 52 (18), 51 (30), 50 (13), 43 (6), 41 (16), 39 (25). R_F 0·55 (chloroform–methanol 99 : 1).

The authors are indebted to colleagues of the Analytical Department, Institute of Chemistry, (Head Dr C. Peciar) for taking IR-, UV-, ORD- and PMR spectra.

REFERENCES

1. Wada H., Shizuri Y., Yamada K., Hirata Y.: Tetrahedron Letters *1971*, 2655.
2. Shizuri Y., Wada H., Sugiura K., Yamada K., Hirata Y.: Tetrahedron Letters *1971*, 2659.
3. Wada H., Shizuri Y., Sugiura K., Yamada K., Hirata Y.: Tetrahedron Letters *1971*, 3131.
4. Pailer M., Streicher W., Leitich J.: Monatsh. Chem. *102*, 1872 (1971).
5. Klásek A., Samek Z., Šantavý F.: Tetrahedron Letters *1972*, 941.
6. Sasaki K., Hirata Y.: J. Chem. Soc. *1972*, 1268.
7. Sugiura K., Shizuri Y., Wada H., Yamada K., Hirata Y.: Tetrahedron Letters *1971*, 2733.
8. Klásek A., Šantavý F., Duffield A. M., Reichstein T.: Helv. Chim. Acta *54*, 2144 (1971).
9. Bishay D. W., Kowalewski Z., Phillipson J. D.: Pharm. Pharmac. *23*, Suppl., 233 (1971).
10. Budzikiewicz H., Djerassi C., Williams D. H.: *Mass Spectrometry of Organic Compounds*, p. 569. Holden-Day, San Francisco 1967.
11. Beroza M.: J. Org. Chem. *28*, 3562 (1963).

Translated by Z. Votický.