Chemical modification of usnic acid
2.* Reactions of (+)-usnic acid with amino acids**

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The condensation of amino acids with (+)-usnic acid involves the 2-positioned acetyl group of the latter and gives derivatives containing an enamine fragment.

Key words: (+)-usnic acid, amino acids, enamines.

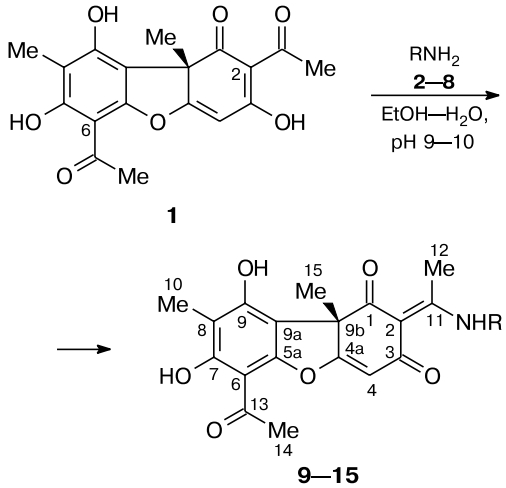
(+)-Usnic acid (**1**), which is a key secondary metabolite of some lichens, is known[2,3] to exhibit antiviral, antibiotic, fungicidal, analgesic, antituberculous, and other types of biological activity. In order to obtain new biologically active compounds, previously,[1] we carried out chemical modification of acid **1** on treatment with perfluoroolefins in the presence of bases and thus synthesized a number of its polyfluoroalkyl and perfluoroalkenyl derivatives. We continued investigation of synthetic transformations of usnic acid (**1**) by performing its condensation with amino acids. It is known[4—6] that introduction of an amino acid fragment into a biologically active molecule may enhance the activity. In addition, upon such a modification, new compounds acquire a carboxy group, which extends the range of possible transformations and can increase water solubility, which is fairly important for biological agents.

No data on the reactions of usnic acid (**1**) with amino acids were reported in the literature. A publication[7] describes reactions of compound **1** with some primary amines, resulting in the condensation products involving the carbonyl groups of either the acetyl fragment at C(2) or two acetyl fragments at C(2) and C(6) to give enamine derivatives (more rarely).

Results and Discussion

We found that the reactions of compound **1** with a number of available amino acids, specifically, glycine (**2**), β-alanine (**3**), L-phenylalanine (**4**), L-valine (**5**), L-leucine (**6**), L-methionine (**7**), and L-serine (**8**), result in enamine derivatives **9**—**15** (Scheme 1).*

Scheme 1



R = C(16)H$_2$C(17)OOH (Gly) (**2**, **9**); C(16)H$_2$C(17)H$_2$C(18)OOH (β-Ala) (**3**, **10**); C(16)H(C(17)OOH)C(18)H$_2$Ph (Phe) (**4**, **11**); C(16)H(C(17)OOH)C(18)H(C(19)H$_3$)(C(20)H$_3$) (Val) (**5**, **12**); C(16)H(C(17)OOH)C(18)H$_2$C(19)H(C(20)H$_3$)(C(21)H$_3$) (Leu) (**6**, **13**); C(16)H(C(17)OOH)C(18)H$_2$C(19)H$_2$SC(20)H$_3$ (Met) (**7**, **14**); C(16)H(C(17)OOH)C(18)H$_2$OH (Ser) (**8**, **15**)

Unlike the condensation with primary amines described in the literature,[7] where the reaction took place on refluxing of reactants in ethanol, the reaction with amino acids proceeded only in an alkaline medium.

* For Part 1, see Ref. 1.

** Dedicated to the memory of Academician N. N. Vorozhtsov on the 100th anniversary of his birth.

* The atom numbering in the compounds is given for assignment of NMR signals and does not always coincide with atom numbering in the IUPAC name.

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We found that compounds **9**—**15** are formed only in the pH range of 9—10. When pH < 9, the condensation does not occur, while at pH >10, usnic acid (**1**) decomposes.[2] The method for isolation of the target product was dictated by the type of amino acid used. Indeed, in the reaction with glycine (**2**), the target condensation product **9** completely precipitated after acidification of the reaction mixture (yield 88%). In the case of amino acids **3**—**7**, products **10**—**14** were distributed between the liquid and solid phases; therefore, their isolation required column chromatography. The yields of products **10**—**13** varied in the range of 47—61%, and the yield of product **14** was only 17%. The moderate yields are due to incomplete conversion of usnic acid (**1**), which did not increase upon the use of either longer reaction times and excess amino acid or upon the variation of the pH. In the reaction with L-serine (**8**), the precipitate consisted of only recovered usnic acid (**1**), while the condensation product **15** was isolated from the filtrate in 29% yield by extraction. No products of condensation of usnic acid with α-alanine, L-lysine, L-tyrosine, L-histidine, and glutamic acid could be isolated, although they were present in the reaction mixtures in trace amounts, as shown by ^{1}H NMR spectroscopy.

The structures of obtained compounds **9**—**15** were established by ^{1}H and ^{13}C NMR and IR spectroscopies and mass spectrometry. The configuration of the C(2)—C(11) double bond in them was suggested based on published data[7] where analogous reaction of usnic acid with ammonia gave a product in which the enamine fragment configuration was established by X-ray crystallography.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on AM-400 and AV-300 Bruker spectrometers operating at 400.13 and 100.61 MHz, respectively. IR spectra were measured on a Specord M-80 spectrometer (in KBr pellets), and mass spectra (ionization electron energy 70 eV) were run on a Finnigan MAT 8200 instrument. The conversion of usnic acid was monitored by ^{1}H NMR spectroscopy. Column chromatography was carried out using silica gel Merck (70—230 μ). Commercially available amino acids were used. Usnic acid was isolated as described previously.[1]

Reactions of usnic acid with amino acids (general procedure). Amino acid (3 mmol) was dissolved in aqueous ethanol (10 mL, 1 : 1, v/v). Potassium hydroxide was added (to adjust pH ~9.5, ≥0.2 g) and the mixture was refluxed for 10 min on a water bath. Then a suspension of (+)-usnic acid (**1**) (344 mg, 1 mmol) in ethanol (5 mL) was added in portions over a period of 30 min and the mixture was refluxed for 3 h on a water bath, pH being maintained at ~9.5. The mixture was cooled and dilute HCl was added to pH ~5; this resulted in precipitation of a light-yellow solid. The subsequent workup of the reaction mixture depended on the type of amino acid used.

6-Acetyl-2-[1-(carboxymethylamino)ethylidene]-7,9-dihydroxy-8,9b-dimethyl-1,2,3,9b-tetrahydrodibenzo[*b*,*d*]furan-1,3-dione (9) was obtained in the reaction with glycine (**2**). The precipitate was collected on a filter, washed with hot water, and dried in air. Yield 88%, m.p. 210 °C (dec.). ^{1}H NMR (DMSO-d_6), δ: 1.65 (s, 3 H, C(15)H$_3$); 1.97 (s, 3 H, C(10)H$_3$); 2.55 (s, 3 H, C(12)H$_3$); 2.64 (s, 3 H, C(14)H$_3$); 4.48 (d, 2 H, C(16)H$_2$, J = 4.6 Hz); 5.90 (s, 1 H, H(4)); 12.21 (s, 1 H, C(9)OH); 13.14 (br.s, 1 H, NH); 13.40 (s, 1 H, C(7)OH). ^{13}C NMR (DMSO-d_6), δ: 7.4 (C(15)); 18.7 (C(12)); 30.9 (C(14)); 31.6 (C(10)); 45.4 (C(16)); 56.3 (C(9b)); 100.8 (C(2)); 101.8 (C(6)); 102.3 (C(4)); 105.1 (C(9a)); 106.3 (C(8)); 155.7 (C(5a)); 157.6 (C(9)); 162.5 (C(7)); 169.2 (C(11)); 172.9 (C(17)); 174.9 (C(4a)); 188.6 (C(3)); 197.5 (C(1)); 201.1 (C(13)). IR, ν/cm^{-1}: 843, 1213, 1288, 1370, 1455, 1552, 1628, 1698, 1734, 3080, 3421. High-resolution MS, found: m/z 401.11090 [M]$^+$. $C_{20}H_{19}NO_8$. Calculated: M = 401.11105.

6-Acetyl-2-[1-(2-carboxyethylamino)ethylidene]-7,9-dihydroxy-8,9b-dimethyl-1,2,3,9b-tetrahydrodibenzo[*b*,*d*]furan-1,3-dione (10) was obtained in the reaction with β-alanine (**3**). The reaction mixture containing a finely dispersed precipitate was extracted three times with ethyl acetate, the extract was dried with $MgSO_4$, and the solvent was removed on a rotary evaporator. Compound **10** was isolated by column chromatography on SiO_2 (gradient elution with $CHCl_3$—AcOEt, 0→50%). Yield 55%, m.p. 105—110 °C. ^{1}H NMR ($CDCl_3$), δ: 1.65 (s, 3 H, C(15)H$_3$); 2.04 (s, 3 H, C(10)H$_3$); 2.61 (s, 3 H, C(12)H$_3$); 2.64 (s, 3 H, C(14)H$_3$); 2.79 (t, 2 H, C(17)H$_2$, J = 6.1 Hz); 3.79 (dt, 2 H, C(16)H$_2$, J = 6.1 Hz, J = 5.8 Hz); 5.80 (s, 1 H, H(4)); 11.71 (s, 1 H, C(9)OH); 13.36 (br.s, 1 H, NH); 13.27 (s, 1 H, C(7)OH). ^{13}C NMR ($CDCl_3$), δ: 7.5 (C(15)); 18.3 (C(12)); 31.2 (C(14)); 32.0 (C(10)); 33.4 (C(17)); 39.4 (C(16)); 57.4 (C(9b)); 101.3 (C(6)); 102.0 (C(4)); 102.3 (C(2)); 104.9 (C(9a)); 108.1 (C(8)); 155.8 (C(5a)); 158.1 (C(9)); 163.5 (C(7)); 173.7 (C(11)); 174.7 (C(17)); 175.5 (C(4a)); 190.5 (C(3)); 198.5 (C(1)); 200.7 (C(13)). IR, ν/cm^{-1}: 668, 1068, 1289, 1372, 1464, 1559, 1626, 1695, 1727, 3023. High-resolution MS, found: m/z 415.12671 [M]$^+$. $C_{21}H_{21}NO_8$. Calculated: M = 415.12670.

6-Acetyl-2-[1-(1-carboxy-2-phenylethylamino)ethylidene]-7,9-dihydroxy-8,9b-dimethyl-1,2,3,9b-tetrahydrodibenzo[*b*,*d*]furan-1,3-dione (11) was obtained in the reaction with L-phenylalanine (**4**). The filtrate was extracted three times with chloroform, and the extract was dried with $MgSO_4$, concentrated *in vacuo*, and combined with the precipitate. Compound **11** was isolated by column chromatography on SiO_2 (gradient elution with $CHCl_3$—AcOEt, 0→50%). Yield 59%, m.p. 240 °C (dec.). ^{1}H NMR ($CDCl_3$), δ: 1.63 (s, 3 H, C(15)H$_3$); 1.97 (s, 3 H, C(10)H$_3$); 2.39 (s, 3 H, C(12)H$_3$); 2.63 (s, 3 H, C(14)H$_3$); 3.15 (dd, 1 H, C(18)H$_2$, J = 14 Hz, J = 7 Hz); 3.29 (dd, 1 H, C(18)H$_2$, J = 14 Hz, J = 4.5 Hz); 5.06 (dd, 1 H, H(16), J = 4 Hz, J = 7 Hz); 5.84 (s, 1 H, H(4)); 7.20—7.27 (5 H arom.); 12.02 (s, 1 H, C(9)OH); 13.35 (br.s, 1 H, NH); 13.35 (s, 1 H, C(7)OH). ^{13}C NMR ($CDCl_3$), δ: 7.5 (C(15)); 18.5 (C(12)); 31.0 (C(14)); 31.6 (C(10)); 38.1 (C(18)); 56.4 (C(9b)); 57.3 (C(16)); 100.8 (C(2)); 101.7 (C(6)); 102.4 (C(4)); 105.0 (C(9a)); 106.4 (C(8)); 127.1 (1 C), 128.4 (2 C), 129.5 (2 C), 135.4 (1 C) (C arom.); 155.7 (C(5a)); 157.6 (C(9)); 162.5 (C(7)); 170.8 (C(11)); 172.9 (C(17)); 174.5 (C(4a)); 188.8 (C(3)); 197.7 (C(1)); 200.9 (C(13)). IR, ν/cm^{-1}: 848, 1070, 1201, 1287, 1372, 1460, 1542, 1632, 1701, 1738, 2926, 3042, 3436. High-resolution MS, found: m/z 491.15982 [M]$^+$. $C_{27}H_{25}NO_8$. Calculated: M = 491.15800.

6-Acetyl-2-[1-(1-carboxy-2-methylpropylamino)ethylidene]-7,9-dihydroxy-8,9b-dimethyl-1,2,3,9b-tetrahydrodibenzo[*b*,*d*]fu-

ran-1,3-dione (12) was obtained in the reaction with L-valine (**5**). The precipitate was filtered off and dried in air, and the product was isolated by column chromatography on SiO_2 (gradient elution with $CHCl_3$—AcOEt, 0→50%). Yield 61%, m.p. 210 °C (dec.). ^{1}H NMR ($CDCl_3$), δ: 1.12, 1.16 (both d, 3 H each, C(19)H_3, C(20)H_3, J = 6.8 Hz); 1.73 (s, 3 H, C(15)H_3); 2.09 (s, 3 H, C(10)H_3); 2.48 (d.sept, 1 H, H(18), J = 4.6 Hz, J = 6.8 Hz); 2.63 (s, 3 H, C(12)H_3); 2.68 (s, 3 H, C(14)H_3); 4.41 (dd, 1 H, H(16), J = 4.6 Hz, J = 8.1 Hz); 5.88 (H(4)); 9.09 (C(17)OOH); 11.78 (br.s, 1 H, C(9)OH); 13.32 (s, 1 H, C(7)OH); 13.90 (br.s, 1 H, NH). ^{13}C NMR ($CDCl_3$), δ: 7.4 (C(15)); 18.7 (C(12)); 17.6, 19.0 (C(19), C(20)); 31.1 (C(14)); 31.4 (C(10)); 38.9 (C(18)); 57.3 (C(9b)); 61.9 (C(16)); 101.3 (C(6)); 102.2, 102.3 (C(2), C(4)); 104.8 (C(9a)); 108.1 (C(8)); 155.7 (C(5a)); 158.0 (C(9)); 163.4 (C(7)); 172.7 (C(17)); 174.6 (C(11)); 175.1 (C(4a)); 190.7 (C(3)); 198.6 (C(1)); 200.7 (C(13)). IR, ν/cm^{-1}: 850, 1067, 1132, 1186, 1287, 1373, 1475, 1555, 1627, 1701, 1741, 2963, 3109. High-resolution MS, found: m/z 443.15820 $[M]^+$. $C_{23}H_{25}NO_8$. Calculated: M = 443.15800.

6-Acetyl-2-[1-(1-carboxy-3-methylbutylamino)ethylidene]-7,9-dihydroxy-8,9b-dimethyl-1,2,3,9b-tetrahydrodibenzo[*b*,*d*]furan-1,3-dione (13) was obtained in the reaction with L-leucine (**6**). The precipitate was filtered off and dried in air, and the product was isolated by column chromatography on SiO_2 (gradient elution with $CHCl_3$—AcOEt, 0→50%). Yield 47%, m.p. 105 °C. ^{1}H NMR ($CDCl_3$), δ: 0.99, 1.05 (both d, 3 H each, C(21)H_3, C(20)H_3, J = 6.4 Hz); 1.74 (s, 3 H, C(15)H_3); 1.85 (m, 1 H, H(19)); 1.94 (m, 2 H, H(18)); 2.10 (s, 3 H, C(10)H_3); 2.65 (s, 3 H, C(12)H_3); 2.69 (s, 3 H, C(14)H_3); 4.53 (m, 1 H, H(16)); 5.87 (s, 1 H, H(4)); 8.26 (br.s, 1 H, C(17)OOH); 11.76 (br.s, 1 H, C(9)OH); 13.34 (s, 1 H, C(7)OH); 13.80 (br.s, 1 H, NH). ^{13}C NMR ($CDCl_3$), δ: 7.0 (C(15)); 18.3 (C(12)); 21.4, 22.2 (C(21), C(20)); 24.4 (C(19)); 30.8 (C(14)); 31.5 (C(10)); 40.9 (C(18)); 54.7 (C(16)); 60.1 (C(9b)); 97.9 (C(2)); 100.9 (C(6)); 101.9 (C(4)); 104.4 (C(9a)); 107.8 (C(8)); 155.3 (C(5a)); 157.7 (C(9)); 163.1 (C(7)); 173.6 (C(11)); 174.3 (C(4a)); 174.4 (C(17)); 190.3 (C(3)); 198.3 (C(1)); 200.3 (C(13)). IR, ν/cm^{-1}: 844, 1064, 1135, 1189, 1289, 1371, 1468, 1553, 1629, 1699, 1745, 2960, 3090. High-resolution MS, found: m/z 457.17270 $[M]^+$. $C_{24}H_{27}NO_8$. Calculated: M = 457.17365.

6-Acetyl-2-{1-[1-carboxy-3-methylthio(propylamino)]ethylidene}-7,9-dihydroxy-8,9b-dimethyl-1,2,3,9b-tetrahydrodibenzo[*b*,*d*]furan-1,3-dione (14) was obtained in the reaction with L-methionine (**7**). The precipitate was filtered off and dried in air, and the product was isolated by column chromatography on SiO_2 (gradient elution with $CHCl_3$—AcOEt, 0→50%). Yield 17%, m.p. 100—102 °C. ^{1}H NMR ($CDCl_3$), δ: 1.69 (s, 3 H, C(15)H_3); 2.04 (s, 3 H, C(10)H_3); 2.12 (s, 3 H, C(20)H_3); 2.35 (m, 2 H, C(18)H_2); 2.64 (s, 3 H, C(12)H_3); 2.65 (m, 2 H, C(19)H_2); 2.67 (s, 3 H, C(14)H_3); 4.82 (m, 1 H, H(16)); 5.85 (s, 1 H, H(4)); 8.69 (br.s, 1 H, C(17)OOH); 11.66 (s, 1 H, C(9)OH); 13.27 (s, 1 H, C(7)OH); 13.61 (br.s, 1 H, NH). ^{13}C NMR ($CDCl_3$), δ: 7.5 (C(15)); 15.3 (C(20)); 18.9 (C(12)); 29.8 (C(18)); 31.3 (C(14)); 31.6 (C(19)); 32.1 (C(10)); 54.9 (C(16)); 57.4 (C(9b)); 101.4 (C(6)); 102.2 (C(4)); 102.8 (C(2)); 104.9 (C(9a)); 108.2 (C(8)); 155.8 (C(5a)); 158.1 (C(9)); 163.5 (C(7)); 172.4 (C(11)); 174.8 (C(17)); 175.5 (C(4a)); 190.6 (C(3)); 198.7 (C(1)); 200.9 (C(13)). IR, ν/cm^{-1}: 841, 1066, 1140, 1190, 1289, 1370, 1464, 1551, 1630, 1700, 1742, 2923, 3080. High-resolution MS, found: m/z 475.12480 $[M]^+$. $C_{23}H_{25}NO_8S$. Calculated: M = 475.13007.

6-Acetyl-7,9-dihydroxy-2-[1-(2-hydroxy-1-carboxyethylamino)ethylidene]-8,9b-dimethyl-1,2,3,9b-tetrahydrodibenzo[*b*,*d*]furan-1,3-dione (15) was obtained in the reaction with L-serine (**8**). According to TLC, the precipitate formed was the recovered usnic acid (**1**). The aqueous filtrate was extracted three times with ethyl acetate, and the extracts were dried with $MgSO_4$ and concentrated *in vacuo*. The residue was compound **15**, yield 29%, m.p. 105—108 °C. ^{1}H NMR ($CDCl_3$), δ: 1.58 (s, 3 H, C(15)H_3); 1.97 (s, 3 H, C(10)H_3); 2.60 (s, 3 H, C(12)H_3); 2.68 (s, 3 H, C(14)H_3); 4.22, 4.34 (both d, AB-system, 2 H, C(18)H_2, J = 11.5 Hz); 4.78 (m, 1 H, H(16)); 5.52 (s, 1 H, H(4)); 11.32 (br.s, 1 H, C(9)OH); 13.24 (s, 1 H, C(7)OH); 13.58 (br.d, 1 H, NH, J = 7.6 Hz). ^{13}C NMR ($CDCl_3$), δ: 7.5 (C(15)); 19.2 (C(12)); 30.9 (C(14)); 31.8 (C(10)); 58.3 (C(16)); 58.6 (C(9b)); 62.9 (C(18)); 101.0, 101.3 (C(6), C(4)); 102.7 (C(9a)); 103.9 (C(2)); 108.4 (C(8)); 155.2 (C(5a)); 157.4 (C(9)); 163.7 (C(7)); 170.2 (C(17)); 174.4, 175.1 (C(11), C(4a)); 190.5 (C(3)); 198.8 (C(1)); 200.3 (C(13)). IR, ν/cm^{-1}: 844, 1065, 1119, 1190, 1286, 1371, 1465, 1557, 1629, 1698, 1745, 2928, 3393. High-resolution MS, found: m/z 431.1222 $[M]^+$. $C_{21}H_{21}NO_9$. Calculated: M = 431.1216.

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