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Isosteviol and some of its derivatives as receptors and carriers of amino acid picrates

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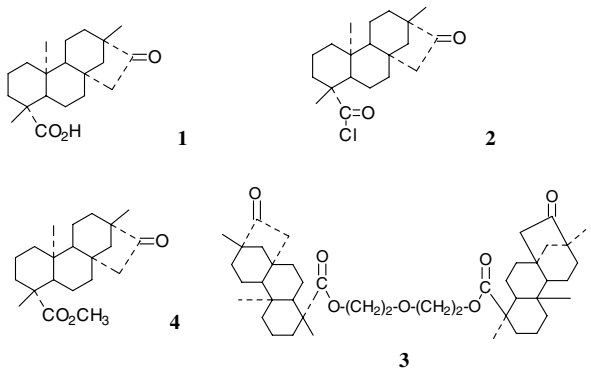
Abstract—The ability of diterpenoid isosteviol **1** (*ent*-16-oxobeyeran-19-oic acid) and some of its derivatives with ester and amide groups to bind amino acid picrates in the course of their transport through a liquid chloroform membrane was observed for the first time. Isosteviol was very competitive with dibenzo-18-crown-6 and *N*,*N*′-bis(isostevioyl)-1,4-phenylenediamine **5** was even more effective in transportation of D,L-tryptophan through a liquid chloroform membrane.

Molecular recognition of biologically important compounds such as amino acids, peptides and proteins is of interest for their selective binding, separation and transport.[1] Many studies have dealt with the design of synthetic receptors, which can bind these substrates, most being focused on variously functionalized macrocycles like crown ethers, aza crown ethers, cyclodextrins, cryptands and calixarenes.[2]

It was discovered recently that diterpenoid isosteviol **1** (*ent*-16-oxobeyeran-19-oic acid) can bind some aromatic molecules forming crystalline inclusion complexes (clathrates) of unusual structures having helical tape assemblies.[3] We have now investigated the ability of isosteviol **1** and diester **3**, methyl ester **4** and diamides **5**–**7** to bind racemic phenylalanine and tryptophan picrates as well as to transport them through a liquid chloroform membrane into a receiving aqueous phase.

Isosteviol **1** was obtained[4] by acid hydrolysis of the crude glycoside fraction extracted from *Stevia rebaudiana* Bertoni. Diester **3** was synthesized by heating isostevioyl chloride[5] **2** with diethylene glycol in the ratio of 2:1 in CCl_4 in the presence of Et_3N.[6] Isosteviol methyl ester **4** was obtained[7] from the reaction of isostevioyl chloride **2** with methanol. The synthesis of diamide **5** was described earlier[8] and diamides **6**–**7** were obtained from isostevioyl chloride **2** by reaction with the corresponding diamines.[9,10] Transport experiments[11] were performed in a similar manner to those with metal picrates.[12] The results are summarized in Table 1.



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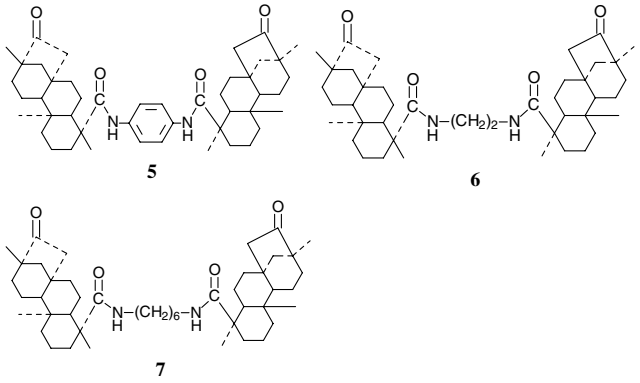

In general, D,L-tryptophan picrate was extracted more efficiently from the source aqueous phase and was transported more efficiently through the liquid chloroform membrane than the less lipophilic D,L-phenylalanine picrate. Isosteviol **1** was highly competitive with dibenzo-18-crown-6, but *N*,*N*′-bis(isostevioyl)-1,4-phenylenediamine **5** surpassed its transport of D,L-tryptophan through the liquid chloroform membrane. It should be noted that the compounds investigated appeared to be extractants rather than carriers of the amino acid picrates. Isosteviol **1** and its methyl ester **4** were practically equal, but only moderate in extracting D,L-phenylalanine and D,L-tryptophan picrates from source aqueous phases (Table 1). Diester **3** extracted D,L-phenylalanine twice as well, probably because there are twice the number of isosteviol moieties in **3** compared to **1** or **4** (while the molar concentration remains the same). We speculate that the role of the lipophilic cavity of compound **3**[6,13] in binding amino acids is insignificant because diamide **5**, with no cavity, extracted D,L-phenylalanine picrate approximately the same as diester **3**, and it is also similar to dibenzo-18-crown-6 (DB18C6) in its ability to extract D,L-tryptophan picrate (Table 1). Diamide **6** extracted D,L-phenylalanine picrate very weakly, and diamide **7** did not extract it at all. There is a rather rough correlation of binding with the length of the spacer. When the spacer consists of six atoms (diamide **6**) or when it consists of ten atoms (diamide **7**), there is no binding. Among compounds with two *ent*-beyeran frameworks only diamide **5**, having a spacer of eight atoms, and diester **3** with a spacer of nine atoms, bind D,L-phenylalanine and D,L-tryptophan picrates. Perhaps these distances are optimal.

In conclusion, further study is required to understand the reasons for binding D,L-phenylalanine and D,L-tryptophan picrates by isosteviol **1**, its methyl ester **4** and some of its diesters and diamides. However, the present preliminary communication establishes that the diterpenoid isosteviol can bind organic molecules not only in the crystal,[3] but in a liquid phase as well. Secondly, some isosteviol diesters (compounds **3** and **4**) and diamide **5** can also transfer amino acid picrates from an aqueous phase to chloroform, *N*,*N*′-bis(isostevioyl)-1,4-phenylenediamine **5** surpasses dibenzo-18-crown-6 in transporting D,L-tryptophan through a liquid chloroform membrane. In other cases, extraction into chloroform prevailed over transport.

Acknowledgements

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References and notes

1. (a) *Comprehensive Supramolecular Chemistry*; Atwood, J. L., Davies, E. D., MacNicol, D. D., Vogtle, F., Murakami, Y., Eds.; Pergamon: Oxford, 1996; Vols. 2 and 4; (b) Zhang, X. X.; Bradshaw, J. S.; Izatt, R. M. *Chem. Rev.* **1997**, *97*, 3313–3361; (c) Miura, Y.; Kimura, S.; Kobayashi, S.; Imanishi, Y.; Umemura, Y. *Biopolymers* **2000**, *55*, 391–395.

Table 1. The transport of D,L-phenylalanine and D,L-tryptophan[a] picrates by isosteviol **1** and its derivatives **3**–**7**, as well as by dibenzo-18-crown-6 (DB18C6) through a liquid chloroform membrane[b]

Amino acid/ligand	Flux *J*, 10^6 M/cm^2 h	Extraction degree, %	The quantity of amino acid held in the chloroform phase, %	The quantity of amino acid transported from the source aqueous phase into the receiving aqueous phase, %
D,L-Phe/**1**	0.08	3.25	2.19	1.06
D,L-Try/**1**	0.38	5.39	0.26	5.13
D,L-Phe/**3**	0.2	6.1	3.4	2.7
D,L-Try/**3**	0.2	7.5	4.8	2.7
D,L-Phe/**4**	0	2.57	2.57	0
D,L-Try/**4**	0.26	5.6	2.0	3.6
D,L-Phe/**5**	0.1	5.5	4.8	0.7
D,L-Try/**5**	0.47	9.6	3.2	6.4
D,L-Phe/**6**	0.06	0.8	0	0.8
D,L-Phe/**7**	0	0	0	0
D,L-Phe/DB18C6	0.15	8.6	6.6	2.0
D,L-Try/DB18C6	0.36	9.8	5.0	4.8

[a] There are no results for transport and extraction of D,L-tryptophan picrates by ligands **6** and **7**, because precipitation occurred at the water/chloroform interface. The structure of the precipitate is not yet known.

[b] Experiments took 6 h; estimated errors are ±2%. In blank experiments (without carrier), there was no observable transport (or extraction) for either of the amino acid picrates.

2. (a) Lehn, J.-M. *Supramolecular Chemistry, Concepts and Perspectives*; VCH: Weinheim, New York, 1995; (b) Buschmann, H.-J.; Mutihac, L.; Jansen, K. *J. Incl. Phenom.* **2001**, *39*, 1–11; (c) Antipin, I. S.; Stoikov, I. I.; Pinkhassik, E. M.; Fitseva, N.; Stibor, I.; Konovalov, A. I. *Tetrahedron Lett.* **1997**, *38*, 5865–5868; (d) Yamaguchi, T.; Nishimura, K.; Shinbo, T.; Sugiura, M. *Bioelectrochem. Bioenerg.* **1988**, *20*, 109–114.
3. (a) Alfonsov, V. A.; Bakaleynik, G. A.; Gubaidullin, A. T.; Kataev, V. E.; Kovyljaeva, G. I.; Konovalov, A. I.; Litvinov, I. A.; Strobykina, I. Yu.; Andreeva, O. V.; Korochkina, M. G. *Mendeleev Commun.* **1999**, *6*, 227–228; (b) Alfonsov, V. A.; Andreeva, O. V.; Bakaleynik, G. A.; Beskrovny, D. V.; Gubaidullin, A. T.; Kataev, V. E.; Kovyljaeva, G. I.; Konovalov, A. I.; Korochkina, M. G.; Litvinov, I. A.; Strobykina, I. Yu.; Musin, R. Z. *Russ. J. Gen. Chem.* **2003**, *73*, 1249–1254; (c) Shagidullin, R. R.; Avvakumova, L. V.; Al'fonsov, V. A.; Bakaleinik, G. A.; Gubaidullin, A. T.; Kataev, V. E.; Kovylyaeva, G. I.; Konovalov, A. I.; Litvinov, I. A.; Strobykina, I. Yu.; Strobykin, S. I.; Andreeva, O. V.; Korochkina, M. G. *Russ. J. Gen. Chem.* **2000**, *70*, 961–969.
4. Alfonsov, V. A.; Bakaleynik, G. A.; Gubaidullin, A. T.; Kataev, V. E.; Kovyljaeva, G. I.; Konovalov, A. I.; Litvinov, I. A.; Strobykina, I. Yu. *Russ. J. Gen. Chem.* **1998**, *68*, 1735–1743.
5. Al'fonsov, V. A.; Bakaleinik, G. A.; Kataev, V. E.; Kovylyaeva, G. I.; Konovalov, A. I.; Strobykina, I. Yu.; Andreeva, O. V.; Korochkina, M. G. *Russ. J. Gen. Chem.* **2000**, *70*, 1318.
6. (a) Alfonsov, V. A.; Bakaleynik, G. A.; Gubaidullin, A. T.; Kataev, V. E.; Kovyljaeva, G. I.; Konovalov, A. I.; Litvinov, I. A.; Strobykina, I. Yu.; Andreeva, O. V.; Korochkina, M. G. *Mendeleev Commun.* **2000**, *5*, 177–178; (b) Alfonsov, V. A.; Andreeva, O. V.; Bakaleynik, G. A.; Beskrovny, D. V.; Gubaidullin, A. T.; Kataev, V. E.; Kovyljaeva, G. I.; Konovalov, A. I.; Korochkina, M. G.; Latypov, Sh. K.; Litvinov, I. A.; Musin, R. Z.; Strobykina, I. Yu. *Russ. J. Gen. Chem.* **2003**, *73*, 1119–1129.
7. Mosetting, E.; Nes, W. R. *J. Org. Chem.* **1955**, *20*, 884–899.
8. Al'fonsov, V.; Kataev, V.; Strobykina, I.; Korochkina, M.; Kovylyaeva, G.; Bakaleinik, G.; Beskrovnyi, D.; Gubaidullin, A.; Litvinov, I.; Musin, R. *Russ. J. Gen. Chem.* **2005**, *75*, 248–253.
9. Synthesis of **6**: A mixture of 1,2-diaminoethane (0.7 mmol) and Et_3N (3 mmol) was added to a solution of isostevioyl chloride **2** (1.5 mmol) in 5 ml of dry CCl_4. The reaction mixture was stirred for 24 h, then was heated to 65 °C, and washed (3×5 ml) with water and dried ($CaCl_2$). Yield: 0.32 g (65%). Mp 253–255 °C. IR spectrum (mineral oil, ν/cm^{-1}): 1251, 1518, 1639 (N–C═O), 1738 (C═O), 3375 (NH). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.49 (s, 2H, NH), 3.36 (s, 4H, NHCH_2) 2.61 (dd, $J = 18.0$, 1.6 Hz, 2H, 15-H_α), 2.2–0.8 (mm, 38H, isosteviol skeleton), 1.17 (s, 6H, 18-H_3), 0.97 (s, 6H, 17-H_3), 0.74 (s, 6H, 20-H_3). MS, m/z 660.4879 (M^+); $C_{42}H_{64}O_4N_2$ requires $M = 660.4866$.
10. Synthesis of **7**: A mixture of 1,6-hexamethylenediamine (0.6 mmol) and Et_3N (2.4 mmol) was added to a solution of isostevioyl chloride **2** (1.2 mmol) in 5 ml of dry CCl_4. The reaction mixture was stirred for 24 h, washed (3×5 ml) with water and dried ($MgSO_4$). Yield: 0.3 g (70%). Mp 130 °C. IR spectrum (mineral oil, ν/cm^{-1}): 1243, 1518, 1639 (N–C═O), 1738 (C═O), 3402 (NH). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.71 (s, 2H, NH), 3.18 (s, 4H, NHCH_2) 2.62 (dd, $J = 18.8$, 1.9 Hz, 2H, 15-H_α), 2.2–0.8 (mm, 38H, isosteviol skeleton), 1.16 (s, 6H, 18-H_3), 0.96 (s, 6H, 17-H_3), 0.76 (s, 6H, 20-H_3). Found: C, 76.64; H, 10.54; N, 3.47%. Calcd for $C_{46}H_{72}O_4N_2$: C, 77.04; H, 10.12; N, 3.90%. MS (MALDI) m/z 717 $[M+H]^+$.
11. The apparatus consists of a cylindrical glass vessel (with an internal diameter 42 mm), and a glass tube (an internal diameter 30 mm) which is positioned into the glass vessel so that the distance between the bottom of the glass tube and the glass vessel bottom equals 0.2–0.5 mm. Twenty milliliters of a 10^{-4} M solution of the carrier in chloroform (liquid chloroform membrane) was added into the cylindrical glass vessel, then 20 ml of a 10^{-4} M solution of amino acid picrate in doubly distilled water (source aqueous phase) was carefully added into the space between the internal wall of the vessel and the external wall of the glass tube. The glass tube itself was filled with 20 ml of doubly distilled water (receiving aqueous phase). Thus, this glass tube separates the two aqueous phases (source and receiving), and the organic layer (liquid chloroform membrane) lies below them and bridges them across the separation by the central glass tube. The chloroform phase was stirred with a magnetic stirrer at 100 rpm for 6 h at 293 ± 1 K. Each experiment was repeated three times and the results were averaged. Amino acid transport was checked by monitoring changes in picrate anion concentration in the source and the receiving phases and in the chloroform by UV measurements ($\lambda = 357$ nm). Additionally, the extraction of tryptophan into chloroform and the receiving aqueous phase was also checked by the appearance of an absorption band at $\lambda = 220$ nm.
12. Kobuke, Y.; Haji, U.; Hriguchi, K. *J. Am. Chem. Soc.* **1976**, *98*, 7414–7419.
13. Kataev, V. E.; Strobykin, S. I.; Timosheva, A. P.; Alfonsov, V. A.; Korochkina, M. G.; Strobykina, I. Yu. *Russ. J. Gen. Chem.* **2005**, *75*, 244–247.