Chemistry of Natural Compounds, Vol. 46, No. 5, 2010

MOLECULAR COMPLEXATION OF THE TRITERPENE GLYCOSIDE HEDERASAPONIN C AND CAFFEINE IN AQUEOUS SOLUTION

L. A. Yakovishin

UDC 547.918:547.857.4:543.42:661.167.7

*Molecular complexation of the triterpene glycoside hederasaponin C [hederagenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl ether] and caffeine in aqueous solution was studied by UV spectroscopy for the first time. The complex composition, which included two glycosides and one caffeine, was determined using isomolar series and molar ratios. The ichthyotoxicity of the complex and its components against* Poecilia reticulata *was studied.*

Keywords: triterpene glycosides, hederasaponin C, caffeine, complexation, clathrate, UV spectroscopy, ichthyotoxicity, *Poecilia reticulata*.

Although caffeine was first discovered in coffee in 1819 by Runge [1], it continues to attract the attention of chemists, biophysicists, and pharmacists due to its high biological activity. Caffeine stimulates the central nervous system, counteracts soporific and narcotic drugs, acts as a diuretic, and intensifies cardiac activity [1, 2]. Herbicidal [3], fungicidal [4], and molluskocidal properties [5] have also been observed for it. Recently the self- and heteroassociation of caffeine has been widely studied. It was found that caffeine in aqueous solutions forms stable associates, among which dimers dominate. Stacking interactions facilitate the aggregation of caffeine [6]. Its interactions with various biologically active compounds, e.g., methylene blue [7], the antibiotic mitoxantrone [8], nucleic acids [9], sodium salicylate and riboflavin [10], sucrose [11], and α- [12] and β-cyclodextrins [11] were previously studied.

Today the complexation of triterpene glycosides with biologically active compounds is of great interest because it represents a method for creating low-dose drugs. Clathrate complexes of glycyrrhizic acid with pyrimidine derivatives, prostaglandins, and cardio-active and psychotropic drugs in addition to complexes of acanthophylloside B isolated from *Acanthophyllum gypsophyloides* roots with prostaglandins were synthesized [13]. Clathrates of Na^+ and K^+ with hederasaponin C in which the cavity was formed by two and three glycoside molecules were found [14]. Complexation of caffeine with hederasaponin C in aqueous solution has not been previously studied by UV spectroscopy.

We used the bisdesmoside triterpene glycoside hederasaponin C (hederacoside C), which is hederagenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-*O*-α-L-arabinopyranosyl-28-*O*-α-L-rhamnopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-*O*-β-D-glucopranosyl ether, as the model glycoside. Hederasaponin C is characteristic of various plant species of the genus *Hedera* L. (ivy) and is the dominant glycoside [15]. It is a component of the cough medicines Hedelix and Prospan, which contain the extract of *H. helix* L. leaves [16].

UV spectroscopy confirmed that intermolecular interactions exist between hederasaponin C and caffeine. As the glycoside concentration increases at constant caffeine concentration ($0.50 \cdot 10^{-4}$ M), the optical density of their solutions increases (hyperchromic effect) (Fig. 1). The absorption maximum of the solutions decreases insignificantly (hypsochromic shift) from 272 (Fig. 1, curve 1) to 270 nm (curve 7). It was also recently reported that a hyperchromic effect occurred upon formation of the clathrate of β-cyclodextrin and caffeine [11].

Sevastopol National Technical University, 99053, Sevastopol, ul. Universitetskaya, 33, Ukraine, e-mail: chemsevntu@rambler.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 629–631, September–October, 2010. Original article submitted February 16, 2010.

 0009-3130/10/4605-0746

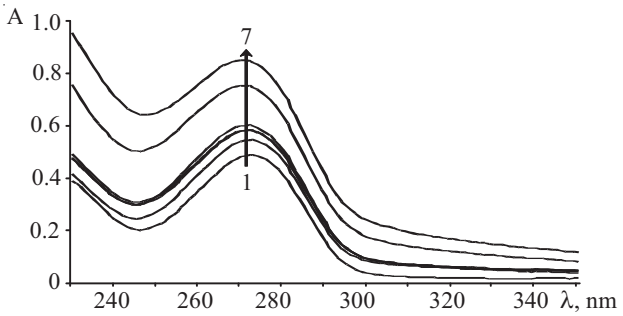

Fig. 1. UV spectra of caffeine solutions ($0.50 \cdot 10^{-4}$ M = const) with different concentrations of hederasaponin C: 0 M (1), 10^{-5} (2), $0.125 \cdot 10^{-4}$ (3), $0.25 \cdot 10^{-4}$ (4), $0.50 \cdot 10^{-4}$ (5), 10^{-4} (6), and $0.75 \cdot 10^{-3}$ (7).

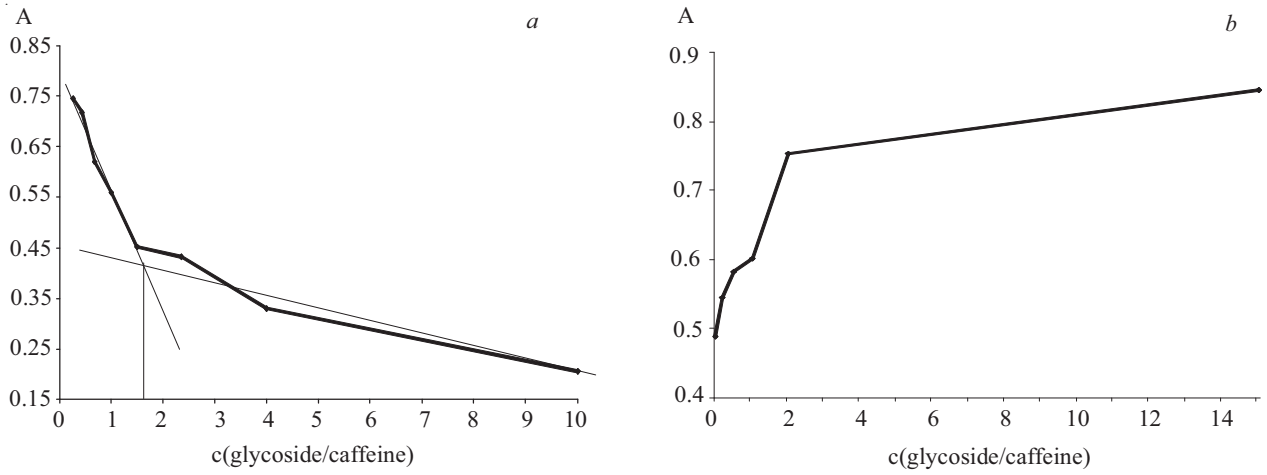

Fig. 2. Optical density A as a function of component ratio of isomolar series at 272 nm: c(glycoside) = 10^{-4} M, c(caffeine) = 10^{-4} M (a) and of glycoside concentration [272 nm, c(caffeine) = $0.5 \cdot 10^{-4}$ M = const] (b).

The composition of the complex was determined by isomolar series and molar ratios (saturation method) [17]. The first method gave a molar ratio ~1.7 (Fig. 2a), which corresponded to a 2:1 glycoside:caffeine complex. The inflection point on the saturation curve (Fig. 2b) was located at a 2.0 glycoside:caffeine concentration ratio. This also confirmed that the stoichiometry of the complex components was 2:1. Such a ratio was obtained for nanostructures of glycyrrhizic acid with several drugs [13].

Considering that hederasaponin C is capable of forming clathrates, the caffeine molecule is obviously situated in a cavity formed by two glycoside molecules. The size of such a cavity, equal to >0.456 nm [14], is sufficient for accommodating a caffeine molecule. The inner diameters of the host cavities in previously prepared inclusion compounds of caffeine with α- and β-cyclodextrins [11, 12] were 0.47–0.53 and 0.60–0.65 nm, respectively [18].

Intermolecular interactions arose between hederasaponin C and caffeine when their solutions were added together. This disrupted the self-association of caffeine, facilitated the formation of caffeine–glycoside heteroassociates, and changed the UV spectra (hyperchromic effect). Considering the structures of the complex components, it was proposed that the clathrate formed between them through hydrophobic interactions of the glycoside aglycon with the heterocyclic system and methyls of caffeine. Obviously caffeine became less hydrated upon formation of the clathrate. This was also observed for the complex of caffeine with β-cyclodextrin [11]. The formation of intermolecular H-bonds involving the pyrimidine C=O and the imidazole N of caffeine and the OH groups of the glycoside monosaccharide units was also possible.

The spectral data indicating that intermolecular interactions occurred between caffeine and the glycoside in aqueous solution were also confirmed by studying the biological activity. Triterpene glycosides are known to exhibit various medical and biological activities, in particular, pronounced toxicity for mollusks and fish [15]. We examined the effects of caffeine, hederasaponin C, and their complex on the fish *Poecilia reticulata* (Poeciliidae) [19]. Low caffeine concentrations

($\leq 0.50 \cdot 10^{-4}$ M) were non-toxic over 3 h. The 100% lethal dose was $0.5 \cdot 10^{-2}$ M caffeine over an average of 11.6 ± 0.1 min; in a mixture containing caffeine ($0.5 \cdot 10^{-2}$ M) and the glycoside (10^{-3} M), over 13.0 ± 0.2 min. A solution of the glycoside ($\leq 10^{-3}$ M) did not exhibit ichthyotoxicity for 30 min because glycosides without a free carboxyl group on C-17 of the aglycon are known to have low toxicity [20]. Therefore, the biological activity of caffeine complexed with hederasaponin C was reduced.

Thus, hederasaponin C formed a molecular complex with caffeine in which the ratio of components was 2:1.

EXPERIMENTAL

We used a glycoside sample obtained from leaves of *H. taurica* Carr. and *H. canariensis* Willd. The isolation and analytical methods have been published [21]. The complex was prepared by mixing aqueous solutions of hederasaponin C and caffeine. The resulting mixtures were held at room temperature (20–22°C) for 40 min with constant stirring. UV spectra were obtained at room temperature (20–22°C) on a Unico UV-Vis 4802 spectrophotometer (USA) in 1-cm quartz cuvettes. We used glycoside and caffeine solutions (10^{-4} M) to formulate the isomolar series. Figure 2 shows the isomolar and saturation curves.

Ichthyotoxicity was tested on *P. reticulata* using solutions of the glycoside and caffeine in distilled water. The action of each separate compound concentration was studied using 20 fish that were placed into solutions of the glycoside, caffeine, and their complex. The incubation time $t_{LD_{100}}$ during which 100% lethality occurred was determined. The confidence range was calculated at reliability level $\alpha = 0.95$.

REFERENCES

1. A. P. Orekhov, *Chemistry of Alkaloids* [in Russian], 2nd Ed., Izd. Akad. Nauk SSSR, Moscow, 1955.
2. M. D. Mashkovskii, *Drugs* [in Russian], in 2 Vol., 13th Ed., Vol. 1, Torsing, Kharkov, 1997, p. 117.
3. S. J. H. Rizvi, V. Rizvi, D. Mukerjee, and S. N. Mathur, *Plant Soil*, **98**, 81 (1987).
4. S. K. Prabhuji, G. C. Srivastava, S. J. H. Rizvi, and S. N. Mathur, *Cell. Mol. Life Sci. (CMLS)*, **39**, No. 2, 177 (1983); S. J. H. Rizvi, V. Jaiswal, D. Mukerji, and S. N. Mathur, *Naturwissenschaften*, **67**, 459 (1980).
5. R. G. Hollingsworth, J. W. Armstrong, and E. Campbell, *Nature*, **417**, 915 (2002); L. Simms and M. Wilson, *Pestic. Outlook*, **13**, No. 6, 270 (2002); H. E. Souza, G. L. G. Soares, and E. C. A. Bessa, *Rev. Bras. Zoociencias Juiz de fora*, **5**, No. 2, 291 (2003).
6. M. Falk, M. Gil, and N. Iza, *Can. J. Chem.*, **68**, 1293 (1990); M. Falk, W. Chew, J. A. Walter, W. Kwiatkowski, K. D. Barclay, and G. A. Klassen, *Can. J. Chem.*, **76**, 48 (1998).
7. S. F. Baranovskii, P. A. Bolotin, and M. P. Evstigneev, *Zh. Prikl. Spektrosk.*, **73**, No. 2, 158 (2006).
8. M. P. Evstigneev, D. A. Veselkov, L. N. Dymant, D. B. Davis, and A. N. Veselkov, *Zh. Strukt. Khim.*, **42**, No. 5, 928 (2001).
9. I. M. Johnson, S. G. B. Kumar, and R. Malathi, *J. Biomol. Struct. Dyn.*, **20**, No. 5, 687 (2003); S. F. Baranovskii, P. A. Bolotin, M. P. Evstigneev, and D. N. Chernyshev, *Zh. Prikl. Spektrosk.*, **76**, No. 1, 143 (2009).
10. S. F. Baranovskii and P. A. Bolotin, *Zh. Prikl. Spektrosk.*, **74**, No. 2, 188 (2007).
11. M. Mejri, A. BenSouissi, V. Aroulmoji, and B. Roge, *Spectrochim. Acta, Part A*, **73**, 6 (2009).
12. I. V. Terekhova, P. V. Lapshev, and O. V. Kulikov, *Koord. Khim.*, **29**, No. 1, 78 (2003).
13. G. A. Tolstikov, L. A. Baltina, V. P. Grankina, R. M. Kondratenko, and T. G. Tolstikova, *Licorice: Biovariety, Chemistry, Use in Medicine* [in Russian], Akad. Izd. Geo, Novosibirsk, 2007, 311; T. G. Tolstikova, A. G. Tolstikov, and G. A. Tolstikov, *Vestn. Ross. Akad. Nauk*, **77**, No. 10, 867 (2007).
14. L. A. Yakovishin, N. I. Borisenko, M. I. Rudnev, E. V. Vetrova, and V. I. Grishkovets, *Khim. Prir. Soedin.*, 45 (2010).
15. K. Hostettmann and A. Marston, *Saponins*, Cambridge Univ. Press, Cambridge, 1995, p. 548.
16. L. A. Yakovishin and V. I. Grishkovets, *Khim. Prir. Soedin.*, 417 (2003); B. M. Zuzuk, R. V. Kutsik, and L. I. Zuzuk, *Provizor*, No. 12, 13 (2003); L. A. Yakovishin, M. A. Vozhzhova, A. L. Kuznetsova, and V. I. Grishkovets, *Zh. Org. Farm. Khim.*, **3**, No. 1 (9), 57 (2005).

17. M. I. Bulatov and I. P. Kalinkin, *Practical Handbook of Photometric Analytical Methods* [in Russian], 5th Ed., Revised, Khimiya, Leningrad, 1986, p. 432.
18. R. Challa, A. Ahuja, J. Ali, and R. K. Khar, *AAPS PharmSciTech*, **6**, No. 2, E329 (2005).
19. I. N. Agekyan, N. V. Belov, and I. L. Kopylov, *Aquarium Encyclopedia* [in Russian], AST, Kharvest, Moscow, Minsk, 2002.
20. M. M. Anisimov and V. Ya. Chirva, *Usp. Sovrem. Biol.*, **6**, No. 3, 351 (1980).
21. A. S. Shashkov, V. I. Grishkovets, A. A. Loloiko, and V. Ya. Chirva, *Khim. Prir. Soedin.*, 363 (1987); V. I. Grishkovets, D. Yu. Sidorov, L. A. Yakovishin, N. N. Arnautov, A. S. Shashkov, and V. Ya. Chirva, *Khim. Prir. Soedin.*, 377 (1996).