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1β-AMINO-2α,3β,5β-TRIHYDROXYCYCLOHEPTANE FROM *PHYSALIS ALKEKENGI* VAR. *FRANCHETI*

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Key Word Index—*Physalis alkekengi* var. *francheti*; Solanaceae; calystegin A_5; 1β-amino-2α,3β,5β-trihydroxycycloheptane; biosynthetic pathway; precursor.

Abstract—A cycloheptane bearing an amino and three hydroxyl groups was isolated from the roots of *Physalis alkekengi* var. *francheti*, along with calystegins A_3, A_5, B_1, B_2, and B_3. By ^{1}H- and ^{13}C-NMR spectra, combined with extensive decoupling experiments, ^{1}H–^{13}C COSY, and NOE enhancements, the structure of this cycloheptane was determined to be 1β-amino-2α,3β,5β-trihydroxycycloheptane. This compound may be a precursor of calystegin A_5 in the biosynthetic pathway or may occur from the enzymic reduction of the 5-aminocycloheptanone derived from the 1,5-bridge scission in calystegin A_5.

INTRODUCTION

The calystegins, a new structural type of polyhydroxylated alkaloids, were first isolated from the roots of *Calystegia sepium* and might act as nutritional mediators of specific plant–bacterium relationships [1]. Calystegins A_3 (**1**), B_1 (**3**), and B_2 (**4**) isolated from this plant were characterized as polyhydroxy-nortropane alkaloids with a bridgehead hydroxyl group [2, 3]. Calystegins have been found in the underground organs and roots of *C. sepium*, *Convolvulus arvensis* (both Convolvulaceae), *Atropa belladonna* (Solanaceae) [1], and in the leaves of *Solanum* sp. and *Datura* sp. (Solanaceae) [4]. In the course of purification of nitrogen-in-the-ring sugars from the leaves and roots of *Morus* sp. (Moraceae), we found calystegins B_2 and C_1 (**6**) [5, 6]. Calystegin C_1 is the first pentahydroxy-nor-tropane described among a large number of the naturally occurring nortropane and tropane alkaloids.

We have recently reported the isolation of calystegins A_3, A_5 (**2**), B_1, B_2, and B_3 (**5**) from the roots of *Physalis alkekengi* var. *francheti* [7]. All calystegins isolated to date have a bridgehead hydroxyl group (or an aminoketal function). This aminoketal system does not occur as an equilibrium mixture of 1-hydroxy-nortropane and 5-aminocycloheptanone but exists entirely in the bicyclic form. From the roots of *P. alkekengi* var. *francheti*, we have isolated the cycloheptane derivative (**7**) which is presumed to be a precursor or a degradation product of calystegin A_5. In this paper, we report the isolation of this cycloheptane derivative and its structure determination.

RESULTS AND DISCUSSION

The alkaloid fraction was obtained by chromatography of the hot water extracts of *Physalis alkekengi* var. *francheti* (roots) on an Amberlite IR-120 (H^+ form) ion-exchange column. The alkaloid fraction was applied to an Amberlite CG-50 (NH_4^+ form) and eluted with water to give **1**, **2**, **3**, **4**, and **5**; compound **7** was eluted with 0.5 M NH_4OH.

High-resolution FAB-mass spectrometry of **7** (glycerol) gave an $[M + H]^+$ ion at *m/z* 162.1134, consistent with a molecular formula of $C_7H_{16}O_3N$. The ^{13}C NMR spectra gave seven resonances at δ 27.5 (*t*, C-7), 34.1 (*t*, C-6), 42.1 (*t*, C-4), 57.5 (*d*, C-1), 69.4 (*d*, C-5), 72.3 (*d*, C-3), and 83.8 (*d*, C-2). The ^{1}H NMR spectral data, combined with extensive decoupling experiments and two dimensional ^{1}H–^{13}C COSY spectral data, defined the complete connectivity of carbon and hydrogen atoms for **7**. The large *J* values ($J_{1,2} = J_{2,3} = 9.1$, $J_{3,4\beta} = 10.6$ Hz) seen in the H-2 and H-3 signals indicate all *trans*-axial orientations of H-1, H-2, and H-3. The NOE effect between H-3 and H-5 or H-1 indicates that the 5-OH group is in a β-orientation. In addition to the large *J* values and NOE effects mentioned above, the definite NOE effects between H-2 and H-4β or H-7β indicate that the seven-membered ring of **7** is in a chair conformation. Therefore, the relative structure of **7** was determined to be 1β-amino-2α,3β,5β-trihydroxycycloheptane.

The tropane alkaloids are a well-recognized group of structurally related natural products and occur mainly in

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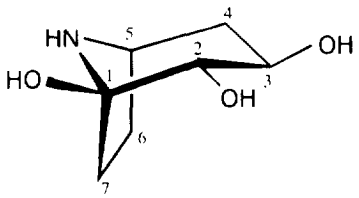
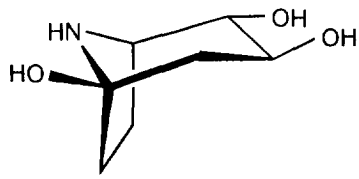
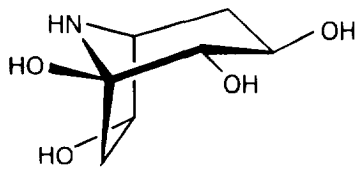


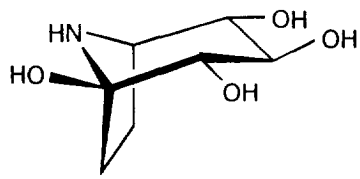
Calystegin A_3 (**1**)



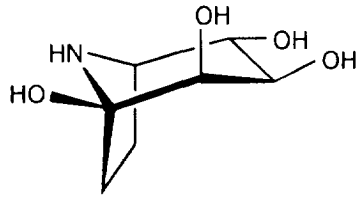
Calystegin A_5 (**2**)



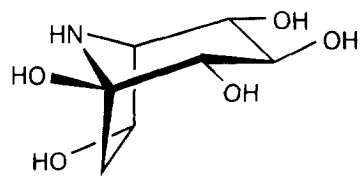
Calystegin B_1 (**3**)



Calystegin B_2 (**4**)



Calystegin B_3 (**5**)



Calystegin C_1 (**6**)

the plant family Solanaceae. A number of hydroxylated tropane alkaloids are hydroxylated at C-3, C-6 and/or C-7 positions of the tropane moeity. In most cases, the C-3 substituent possesses an axial configuration. However, in the calystegins isolated to date, hydroxyl substitution has been shown to occur at the C-1 (bridgehead) position, and concominantly at C-2, C-3, C-4 and/or C-6. Furthermore, the configuration at C-3 in all calystegins (A_3, A_5, B_1, B_2, B_3, and C_1) is equatorial. A unique feature of calystegins lies in the presence of an aminoketal system at the bridgehead position. This aminoketal system does not exist as an equilibrium mixture of 1-hydroxynortropane and 5-aminocycloheptanone but exists entirely in the bicyclic form. One of the reasons for this equilibrium being shifted toward one of these forms is presumed to be the nature of substituents on the seven-membered ring [8]. Among the large number of tropane and nortropane alkaloids, calystegins, physoperuvine [9], and 1-hydroxytropacocaine [10] are the only ones bearing a bridgehead hydroxyl group. The formation of the nortropane ring in calystegins appears to be different from that of the tropane ring in hyoscyamime and scopolamine [11, 12], because it seems most likely that the 1-hydroxynortropane skeleton results from the intramolecular cyclization of the corresponding 5-aminocycloheptanone (Fig. 2). Therefore, 1β-amino-2α,3β,5β-trihydroxycycloheptane (**7**) might be a precursor on the biosynthetic pathway that leads to calystegin A_5, as shown in Fig. 2, or it may be a product of the enzymic reduction of the 5-aminocycloheptanone derived from the 1,5-bridge scission in calystegin A_5.

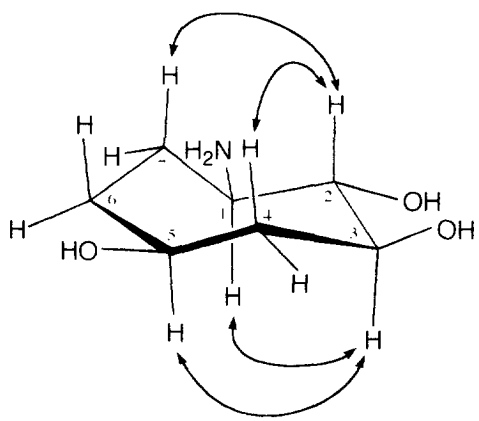


Fig. 1. NOE effects of 1β-amino-2α,3β,5β-trihydroxycycloheptane (**7**).

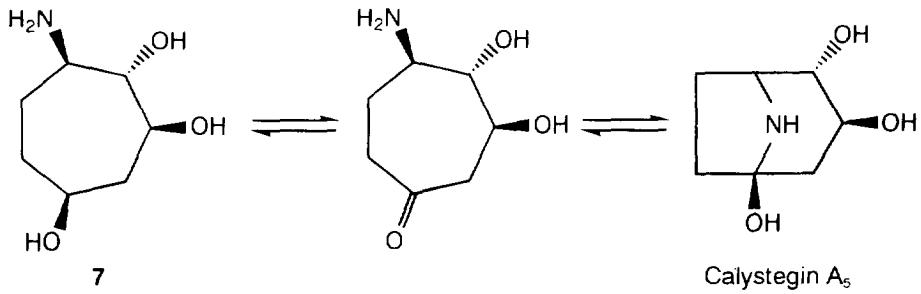


Fig. 2. The proposed biosynthetic or degradative pathway of calystegin A_5.

EXPERIMENTAL

General. Alkaloids were chromatographed by HPTLC silica gel-60F_{254} (E. Merck) using solvent system *n*-PrOH–AcOH–H_2O (4:1:1), with detection by spraying with the chlorine-*o*-tolidine reagent. Optical rotation was measured with a Jasco DIP-370 digital polarimeter. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Jeol JNM-GX 400 spectrometer as indicated in D_2O using sodium 3-(trimethylsilyl)propionate (TSP) as an internal standard. Mass spectrometry data were measured on a Jeol JMS-SX 102A spectrometer.

Isolation of alkaloids. The alkaloid fraction was obtained by chromatography of the hot H_2O extracts of the roots (5 kg) of *P. alkekengi* var. *francheti* on an Amberlite IR-120 (H^+ form) ion-exchange column as described in the ref. [7]. The alkaloid fr. was applied to Amberlite CG-50 (NH_4^+ form) and eluted with H_2O to give the mixture of calystegins A_3, A_5, B_1, B_2, and B_3, while compound **7** was eluted with 0.5 M NH_4OH. Crude compound **7** was further chromatographed on a Dowex 1-X2 column (1 × 56 cm, OH^-) with H_2O as eluant (fr. size 5 ml) to give a pure sample (11 mg).

1β-Amino-2α,3β,5β-trihydroxycycloheptane (**7**). Oil. $[\alpha]_D$ −6.1° (H_2O, 0.32). HRFAB-MS (glycerol) *m/z*: 162.1134 $[M+H]^+$ ($C_7H_{16}O_3N$ requires 162.1130). ^{1}H NMR: δ 1.67 (*m*, 2H, H-7α, 7β), 1.74 (*m*, 1H, H-4β), 1.80 (*m*, 2H, H-6α,6β), 2.10 (*ddd*, 1H, $J_{3,4\alpha}$ = 1.8, $J_{4\alpha,5}$ = 5.5, $J_{4\alpha,4\beta}$ = 13.6 Hz, H-4α), 2.74 (*m*, 1H, H-1), 3.19 (*t*, 1H, $J_{1,2} = J_{2,3}$ = 9.1 Hz, H-2), 3.49 (*ddd*, 1H, $J_{2,3}$ = 9.1, $J_{3,4\alpha}$ = 1.8, $J_{3,4\beta}$ = 10.6 Hz, H-3), 4.08 (*m*, 1H, H-5). ^{13}C NMR: δ 27.5 (*t*, C-7), 34.1 (*t*, C-6), 42.1 (*t*, C-4), 57.5 (*d*, C-1), 69.4 (*d*, C-5), 72.3 (*d*, C-3), 83.8 (*d*, C-2).

REFERENCES

1. Tepfer, D. A., Goldmann, A., Pamboukdjian, N., Maille, M., Lepingle, A, Chevalier, D., Denarie, J. and Rosenberg, C. (1988) *J. Bacteriol.* **170**, 1153.
2. Ducrot, P. H. and Lallemand, J. Y. (1990) *Tetrahedron Letters* **31**, 3879.
3. Goldmann, A., Milat, M. L., Ducrot, P. H., Lallemand, J. Y., Maille, M., Lepingle, A., Charpin, I. and Tepfer, D. (1990) *Phytochemistry* **29**, 2125.
4. Nash, R. J., Rothschild, M., Porter, E. A., Watson, A. A., Waigh, R. D. and Waterman, P. G. (1993) *Phytochemistry* **34**, 1281.
5. Asano, N., Tomioka, E., Kizu, H. and Matsui, K. (1994) *Carbohydr. Res.* **253**, 235.
6. Asano, N., Oseki, K., Tomioka, E., Kizu, H. and Matsui, K. (1994) *Carbohydr. Res.* **259**, 243.
7. Asano, N., Kato, A., Oseki, K., Kizu, H. and Matsui, K. (1995) *Eur. J. Biochem.* **229**, 369.
8. Boyer, F. D., Ducrot, P. H., Henryon, V., Soulie, J. and Lallemand, J. Y. (1992) *Synlett.* **1992**, 357.
9. Pinder, A. R. (1982) *J. Org. Chem.* **47**, 3607.
10. Moore, J. M., Hays, P. A., Cooper, D. A., Casale, J. F. and Lydon, J. (1994) *Phytochemistry* **36**, 357.
11. Lounasmaa, M. (1988) *Alkaloids* **33**, 1.
12. Robins, R. J., Walton, N. J., Parr, A. J., Aird, E. L. H., Rhodes, M. J. C. and Hamill, J. D. (1994) *Rec. Adv. Phytochem.* **28**, 1.