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Structure of the *Calystegines*: new alkaloids of the nortropane family.

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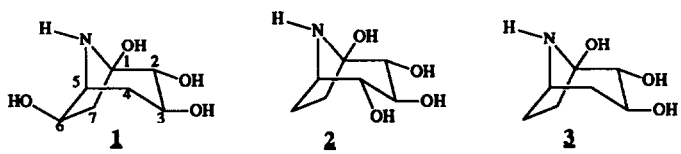
ABSTRACT: *Three new alkaloids have been isolated from* Calystegia sepium*; their structures have been established from their* ^1H *and* ^{13}C *N.M.R. spectra, and confirmed by the synthesis of model compounds.*

In a quest towards a better understanding of how plants interact with their environment, and more precisely with soil bacteria, three new alkaloids, called *calystegines* B_1, B_2, and A_3 have been isolated from the roots of *Calystegia sepium*[1] ; they are supposed to enhance the growth of the *Rhizobium* species of bacteria. We wish to present here their structure elucidation and confirmation by the synthesis of model compounds.

After extraction and HPLC purification, *calystegines* B_1, B_2, and A_3[1] were subjected to high resolution mass spectrometric analysis, leading to molecular formulae: $C_7H_{13}O_3N$ (M=159.0895) with 4 exchangeable hydrogens for *calystegine* A_3 (C.I. with ND_3), and $C_7H_{13}O_4N$ (M=175.0844) with 5 exchangeable hydrogens for *calystegines* B_1 and B_2.



Scheme 1

The ^{13}C N.M.R. spectrum of *calystegine* B_1 revealed 1 quaternary carbon (δ=93.0 ppm), 4 methine carbons (δ=60.0, 70.0, 73.0, 82.0 ppm) and 2 methylene carbons (δ=25.0, 32.0 ppm). The ^{13}C N.M.R. spectra of *calystegines* B_2 and A_3 also displayed also 1 quaternary carbon at 93 ppm similar to that observed in *calystegine* B_1, and also 4 methine carbons (δ=58.0, 76.5(2C), 80.3 ppm) and 2 methylene carbons (δ=24.2, 31.0 ppm) for *calystegine* B_2, 3 methine carbons (δ=54.0, 72.5, 82.5 ppm) and 3 methylene carbons (δ=29.1, 31.0, 42.5 ppm) for *calystegine* A_3.

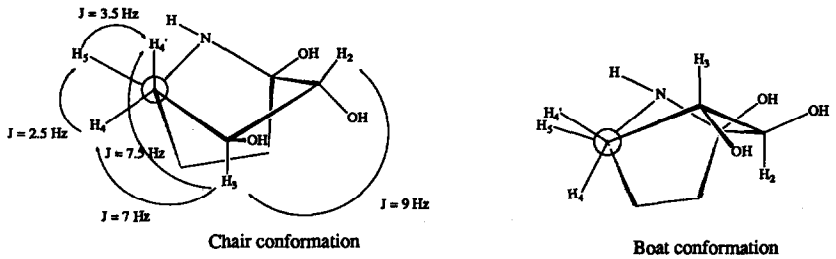
^1H N.M.R. studies at high field[2] (400 MHz) with extensive ^1H-^1H decoupling experiments lead us to propose structures 1, 2 and 3 for *calystegines* B_1, B_2, and A_3 (see *Scheme 1*).

In *calystegine* B_1 2 isolated proton spin systems were identified:
a first spin system of 5 coupled protons was assigned to H-2, H-3, H-4, H-4' and H-5; the second spin system of 3 protons was assigned to H-6, H-7 and H-7'. A small long range coupling constant can be detected between H-2 and H-7 (J=1.5 Hz). The stereochemistry at C-6 is in agreement with the observation that the 2 vicinal protons H-5 and H-6 do not display any coupling, due to a dihedral angle close to 90° (bridgehead and endo protons). The coupling constant measurements indicated that the 3 protons on C-2, C-3 and C-4, bearing the hydroxy groups, are axial (J=7.5-9 Hz).

The structure of *calystegine* B_2 has been elucidated in a similar manner, noting that C-6 was not bearing an hydroxy group since H-5 displays 2 coupling constants with H-4 and H-6 respectively ($J_{4,5} = 3.5Hz$; $J_{5,6} = 9Hz$).

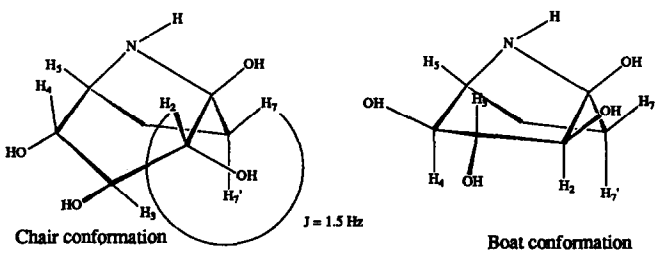
The elucidation of the structure of *calystegine* A_3 has been achieved in the same manner.

It is important to note that the six-membered ring of *calystegines* has a chair conformation, in view of the fact that, in *calystegine* B_1, proton H-5 displays 2 coupling constants for H-4 and H-4' respectively ($J_{4,5}$=2.5 Hz; $J_{4',5}$=3.5 Hz), which can only appear in a chair conformation: a boat conformation would provide a dihedral angle close to 90° between C-5-H-5 and C-4-H-4' bonds, and another one close to 0° between C-5-H-5 and C-4-H-4 bonds, as shown in *Scheme 2.*



Scheme 2

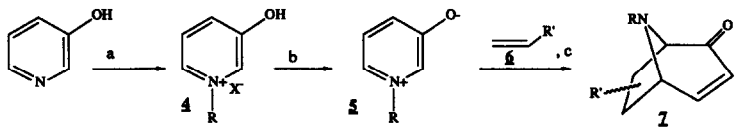
An additional proof is provided by observation of a long range coupling constant between H-2 and H-7, which can only appear in a chair conformation of the six-membered ring. (see *Scheme 3.*)[3]



Scheme 3

Confirmation of structure

In order to confirm these structures, we have prepared some products, having the tropane skeleton by the dipolar addition of 3-oxo pyridinium betaines to activated olefins[4]. The results are summarized in *Scheme 4*.



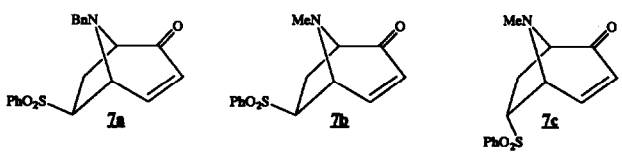
a: CH_3I or BnBr, refluxing PrOH. b: MeONa, MeOH. c: refluxing THF.

R=	6*R'*	*nb of isomers*	*react. time*	*yield %* 4- 7
Me	CN	2	36 h.	40
	SO_2Ph	2	14 h.	68
	CO_2Me	4	36 h.	15
Bn	CN	2	3 d.	48
	SO_2Ph	1	16 h.	72
	CO_2Me	4	36 h.	20
	$SOPh$	1	4 d.	39

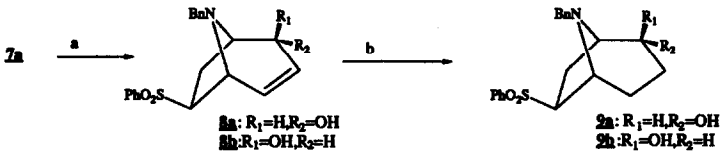
Scheme 4

A similar approach to the tropane skeleton has recently been described by Koizumi *et al.*[5].

The stereoselectivity observed in this reaction must be related to the difference in steric hindrance provided by the R and R' groups. When phenyl-vinyl sulfone was used as activated olefin, only one isomer is obtained when R is a benzyl group: structure **7a** [6], with an *exo* substituent was assigned to this adduct, according to the fact that no coupling constant is detected between H-5 and H-6_{endo}. Two isomeric compounds **7b** and **7c**[7] are obtained when a methyl group is the R substituent, corresponding respectively to the *exo* and the *endo* substituted cycloadducts. This provides a convenient proof of the stereochemistry of carbon C-6 of *calystegine* B_1, since H-5 and H-6_{exo} of **7c** display a coupling constant of 6 Hz, which is in agreement with that observed for H-5 and H-6 of *calystegine* B_2.



Reduction of **7a** affords two isomeric allylic alcohols **8a** and **8b**, which, upon catalytic hydrogenation, yield a mixture of the two saturated alcohols **9a** and **9b**. Equatorial alcohol **9a** displays a coupling constant of 2.5 Hz between H-1 and H-2, in agreement with that detected in the *calystegines* ($J_{4,5}$=3.5 Hz). An additional proof comes from the detection in alcohol **9a** of a small long range coupling constant between H-2 and H-7_{exo} (J=1Hz), which does not appear in compound **9b**[8].



a: DIBAH, THF; 70%. b: H_2, Pd/C, EtOH; 80%.

Scheme 5

References and notes

1: D. Tepfer, A. Goldmann, N. Pamboukdian, M. Maille, A. Lepingle , D. Chevalier, J. Denarié and C. Rosenberg: *J. of Bact.*, **1988**, *170*, 1153.

2: CALYSTEGINE B_1: ^{13}C:δ (ppm) :25.0, 32.0($C-4, C-7$); 60.0($C-5$); 70.0, 73.0, 82.0($C-2, C-3, C-6$); 93.0($C-1$)
^1H:δ(ppm) :3.95($H-6$); 3.3($H-3$); 3.2($H-2$); 3.12($H-5$); 2.35($H-7'$); 1.88($H-4'$); 1.3($H-4, H-7$)
$J_{3,2} = 9Hz$; $J_{3,4'} = 7.5Hz$; $J_{3,4} = 7Hz$; $J_{4',4} = 13Hz$; $J_{5,4} = 2.5Hz$; $J_{5,4'} = 3Hz$; $J_{2,7} = 1.5Hz$ $J_{6,7} = 7Hz$; $J_{6,7'} = 8Hz$; $J_{7,7'} = 15Hz$
Specific rotation not avalaible.
CALYSTEGINE B_2: ^{13}C:δ (ppm) :24.2, 31.0($C-6, C-7$); 58.0($C-5$); 76.5($2C$), 80.3($C-2, C-3, C-4$); 93.0($C-1$)
^1H:δ(ppm) :3.41($H-4$); 3.25($H-2$); 3.17($H-3$); 3.12($H-5$); 1.8($H-6, H-6'$); 1.58($H-7$); 1.37($H-7'$)
$J_{4,3} = 8.5Hz$; $J_{4,5} = 3.5Hz$; $J_{2,3} = 9Hz$; $J_{5,6} = 9Hz$; $J_{6,7} = 7Hz$; $J_{6,7'} = 9Hz$; $J_{7,7'} = 12Hz$; $J_{6,6'} = 11Hz$; $J_{2,7'} = 1.5Hz$
$[\alpha]_D$= 2,9° (c = 0,2 H_2O)
CALYSTEGINE A_3: ^{13}C:δ (ppm) :29.1, 31.0, 42.5($C-4, C-6, C-7$); 54.0($C-5$); 72.5, 82.5($C-2, C-3$); 93.0($C-1$)
^1H: δ(ppm) :1.4 – 1.9($6H$)($H-4, H-4', H-6, H-6', H-7, H-7'$); 3.3($H-2$); 3.4($H-5$); 3.6($H-3$)
Specific rotation not avalaible.

3: for general reviews on the tropane alkaloids see G. Fodor and R. Dharanipragada: *Nat. Prod. Rep.*, **1986**, *3*, 181 and references cited therein; G. Fodor in "The Alkaloids" ed. by R. H. F. Manske vol. VI,145 and vol. IX, 269, Academic press.

4: C. Y. Ishag, K. J. Fisher, B. E. Ibrahim, G. M. Iskander and A. R. Katritsky: *J. Chem. Soc. Perkin Trans. I*, **1988**, 917, and references cited therein.

5: T. Takahashi, T. Hagi, K. Kitano, Y. Takeuchi and T. Koizumi: *Chem. Lett.*, **1989**, 593.

6: 7a: N.M.R. ^1H δ (ppm) :7.05($dd, H-4$); 6.1($dd, H-3$); 4.17($d, H-5$); 3.75 (AB: J=12.5 Hz, CH_2Ph); 3.6($dd, H-1$); 3.55($dd, H-6$);2.8($ddd, H-7$);2.0($dd, H-7'$)
$J_{3,4} = 10Hz$; $J_{4,5} = 5Hz$; $J_{3,1} = 1.5Hz$; $J_{1,7} = 7.5Hz$; $J_{7,7'} = 15Hz$; $J_{6,7} = 5Hz$; $J_{6,7'} = 9Hz$

7: 7c: N.M.R. ^1H δ (ppm) : 6.65($dd, H-4$); 6.05($dd, H-3$); 4.35($d, H-5$); 3.62($dd, H-6$); 3.55($dd, H-1$); 2.75($ddd, H-7$); 1.9($dd, H-7'$)
$J_{3,4} = 10Hz$; $J_{4,5} = 5Hz$; $J_{3,1} = 1.5Hz$; $J_{1,7} = 7Hz$; $J_{7,7'} = 15Hz$; $J_{6,7} = 5Hz$; $J_{6,7'} = 8Hz$
7b N.M.R. ^1H δ (ppm) :7.0($dd, H-4$); 5.95($dd, H-3$); 3.85($m, 2H; H-1, H-6$); 3.45($t, H-5$); 2.65($ddd, H-7$); 2.1($dd, H-7'$)
$J_{3,4} = 10Hz$; $J_{4,5} = 5Hz$; $J_{3,1} = 1.5Hz$; $J_{7,7'} = 12Hz$; $J_{7',6} = 10Hz$; $J_{7,6} = 5Hz$; $J_{5,6} = 6Hz$; $J_{1,7} = 6Hz$

8: 9a: N.M.R. ^1H δ (ppm) :4.05 – 3.7($AB : J = 12.5Hz, CH_2Ph$); 3.8($m, H-5$); 3.55($t, H-6$);3.5 ($m, H-2$); 3.4($m, H-1$) 2.55($ddd, H-7$); 1.95($m, H-4_{ax}$); 1.75($dd, H-7'$); 1.6($m, H-3_{eq}$); 1.4($m, H-3_{ax}$); 1.35($m, H-4_{eq}$)
$J_{4ax,5} = 3Hz$; $J_{4eq,5} = 5Hz$; $J_{4ax,4eq} = 14Hz$; $J_{1,2} = 2.5Hz$; $J_{1,7} = 5Hz$; $J_{6,7'} = 6Hz$; $J_{6,7} = 6Hz$; $J_{1,7} = 5Hz$; $J_{3eq,4ax} = 4Hz$; $J_{3ax,4ax} = 12Hz$; $J_{2,3eq} = 3Hz$; $J_{2,7} = 1Hz$
9b: N.M.R. ^1H δ (ppm) :4.0 – 3.7($AB : J = 12.5Hz, CH_2Ph$); 3.75($m, H-5$); 3.5($t, H-6$);3.45($m, H-2$); 3.3($m, H-1$) 2.3($ddd, H-7$); 2.15($dd, H-7'$); 1.85($m, H-4_{ax}$); 1.6($m, H-3_{eq}$); 1.4($m, H-3_{ax}$); 1.35($m, H-4_{eq}$)
$J_{4ax,5} = 3Hz$; $J_{4eq,5} = 5Hz$; $J_{4ax,4eq} = 14Hz$; $J_{1,2} = 2.5Hz$; $J_{1,7} = 5Hz$; $J_{6,7'} = 6Hz$; $J_{6,7} = 6Hz$; $J_{1,7} = 5Hz$; $J_{3eq,4ax} = 4Hz$; $J_{3ax,4ax} = 12Hz$; $J_{2,3ax} = 4.5Hz$

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