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Biological Activities of the Nortropane Alkaloid, Calystegine B_2, and Analogs: Structure−Function Relationships

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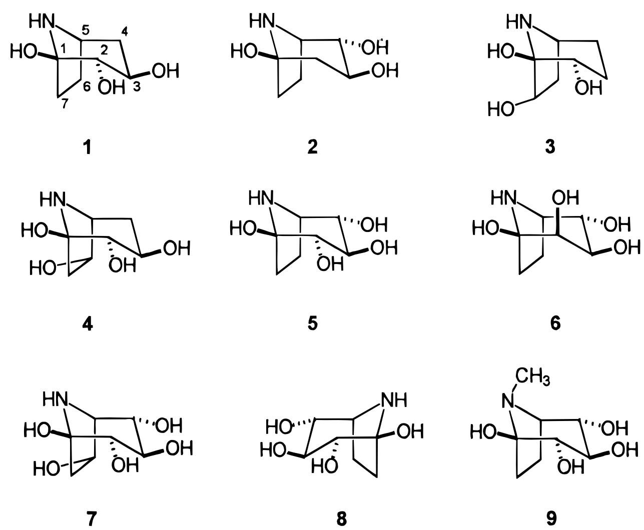
Calystegines, polyhydroxy nortropane alkaloids, are a recently discovered group of plant secondary metabolites believed to influence rhizosphere ecology as nutritional sources for soil microorganisms and as glycosidase inhibitors. Evidence is presented that calystegines mediate nutritional relationships under natural conditions and that their biological activities are closely correlated with their chemical structures and stereochemistry. Assays using synthetic (+)- and (−)-enantiomers of calystegine B_2 established that catabolism by *Rhizobium meliloti*, glycosidase inhibition, and allelopathic activities were uniquely associated with the natural, (+)-enantiomer. Furthermore, the *N*-methyl derivative of calystegine B_2 was not catabolized by *R. meliloti*, and it inhibited α-galactosidase, but not β-glucosidase, whereas the parent alkaloid inhibits both enzymes. This *N*-methyl analog therefore could serve to construct a cellular or animal model for Fabry's disease, which is caused by a lack of α-galactosidase activity.

Calystegines[1] are novel allelochemicals: tropane alkaloids characterized by the absence of *N*-methylation (a nortropane ring system), a high degree of hydroxylation, and an unusual aminoketal functionality at the bridgehead position.[2,3] Seven calystegines, differing in the number, position, and stereochemistry of the hydroxyl group substituents on the tropane ring, have been isolated from certain species in the plant families Convolvulaceae and Solanaceae,[2,4−9] and several have been shown to occur in *Morus* species (family Moraceae).[10,11] This wide distribution among unrelated plant families suggests that calystegines may ultimately be found in additional taxa. The known members of the class have been subdivided into three groups on the basis of the number of hydroxyl groups present, namely calystegines A, B, and C. Calystegines A_3 (**1**), A_5 (**2**), and A_6 (**3**) each have three, calystegines B_1 (**4**), B_2 (**5**), and B_3 (**6**) have four, and calystegine C_1 (**7**) has five hydroxyl groups, respectively (Chart 1). The absolute configuration has been established for calystegine B_2 as (1*R*,2*S*,3*R*,4*S*,5*R*)-1,2,3,4-tetrahydroxynortropane (**5**) by stereospecific synthesis of the natural, (+)-enantiomer and the corresponding (−)-enantiomer **8**.[12−14] Calystegine A_3 was synthesized as a racemic mixture[15] and as the (+)- and (−)-enantiomers,[16] confirming the general structure, but no comparison of the specific rotations with that of the natural alkaloid has been made. However, it is reasonable to presume from biosynthetic considerations that the configuration of all of the calystegines is analogous to that of calystegine B_2. Although the calystegines have been isolated from several Solanaceous species known to elaborate typical tropane alkaloids, no polyhydroxytropanes such as *N*-methylcalystegine B_2 (**9**) have been isolated to date.

Chart 1



Plants communicate among themselves and with other organisms by releasing secondary metabolites that participate in signaling networks essential to maintaining ecological order. The relative abundance of calystegines in subterranean organs and their exudation by *Agrobacterium rhizogenes* transformed root cultures grown *in vitro*, together with their utilization as a carbon and nitrogen source by rare, calystegine-catabolizing (Cac^+) bacteria such as *Rhizobium meliloti* 41,[17] indicates that these alkaloids may have a role as nutritional mediators in the rhizosphere. Calystegine catabolism in *R. meliloti* 41 is conferred by plasmid Rme41a,[17] of which approximately 30−40 kb is implicated in the process.[18] The calystegine catabolic region appears to be similar in complexity to those of the T0L plasmids of *Pseudomonas putida*[19] but the catabolic enzymes have not been described.

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The finding that calystegines, in common with polyhydroxypyrrolizidine and -indolizidine alkaloids, are specific and potent inhibitors of glycosidases[9,11,20] suggests that they might also act as allelopathic substances. They could thus confer a competitive advantage on the producing plants, on catabolic soil bacteria that are able to detoxify and utilize them, and on plants sensitive to calystegines, but which associate with catabolic bacteria for detoxification.[18] As with other alkaloidal glycosidase inhibitors, there is evidence that they may also be toxic to mammals,[5,21] causing neurological disorders in livestock and contributing to predator defense mechanisms in *Lepidoptera* larvae feeding upon plants that produce them.[8] In this paper, we establish that Cac^+ bacteria associate with plants that produce calystegines (Cas^+), suggesting that calystegines function as nutritional mediators under natural conditions. We also demonstrate the allelopathic properties of calystegine B_2 and show that three biological functions (bacterial catabolism, glycosidase inhibition, and allelopathic activities) are constrained by the stereochemistry of the alkaloid.

Results and Discussion

It was previously proposed[17] that calystegines produced in underground organs of the morning glories or bindweeds, *Calystegia sepium* and *Convolvulus arvensis* (Convolvulaceae), and in *Atropa belladonna* (Solanaceae) may provide an exclusive carbon and nitrogen source to soil bacteria, such as *R. meliloti* 41, that carry calystegine catabolism (*cac*) genes. If this were true, the rhizospheres of these plants should contain Cac^+ bacteria. The frequency of Cac^+ bacteria isolated from the rhizospheres of plants (Cas^+) that produce calystegines, and from plants (Cas^-) that do not, was therefore compared. Of 51 isolates from calystegine-producing *C. sepium* and *C. arvensis*, 11 (22%) were Cac^+. Among these, two were Gram-negative and identified as Pseudomonads (Institut Pasteur, Paris), one was taxonomically undetermined, and the other was a *Pseudomonas fluorescens*. No Cac^+ bacteria were found among the 42 colonies isolated from two species that do not produce calystegines (a dicot, *Alliaria petiolata*, and a monocot, a domesticated lawn grass).

In order to compare the cac regions from *R. meliloti* and from wild Cac^+ rhizosphere bacteria, whole cell DNA was extracted from *R. meliloti* 41 and from three wild Cac^+ bacteria. These samples were probed with the cac region from pRme41a, represented by pGMI4103, producing no evidence for sequence similarity (Figure 1).

The most abundant calystegines, A_3 (**1**), B_1 (**4**), and B_2 (**5**), occurring in *C. sepium*, differ in the number and the position of the hydroxyl group substituents on the nortropane ring, yet all three are catabolized by Cac^+ soil bacteria. In order to further define the significance of stereochemistry to biological activity, the catabolism of synthetic calystegine B_2 (+)- and (−)-enantiomers[14,15] by *R. meliloti* 41, a Cac^+ strain, was compared. Only the natural form, (+)-calystegine B_2, was degraded (Figure 2). Furthermore, the *N*-methyl derivative of (+)-calystegine B_2 was not catabolized (Figure 2), nor was the polyhydroxy indolizidine alkaloid, castanospermine, indicating the necessity for the presence of a secondary, rather than tertiary, nitrogen atom, in addition to the hydroxyl groups, for degradation to occur.

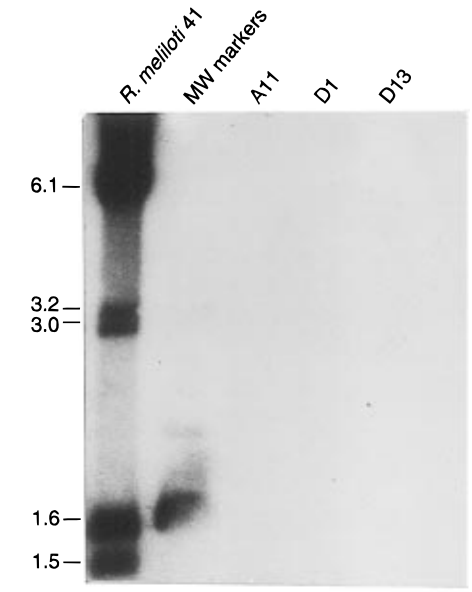

Figure 1. Results of a search for sequence similarity between *cac* genes in *R. meliloti* and wild Cac^+ bacteria. Lane 1: positive control, whole cell DNA from *R. meliloti* 41. Lane 2: molecular weight markers showing limited hybridization to the probe. Lanes 3−5: DNA extracted from three wild, Cac^+ bacteria and cut with HindIII before gel electrophoresis, showing no hybridization.

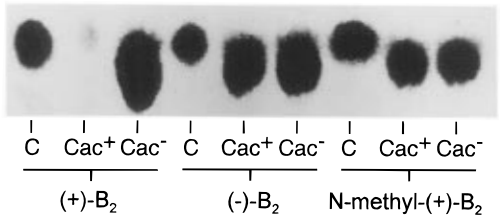

Figure 2. Catabolism of (+)-calystegine B_2, (−)-calystegine B_2, and *N*-methyl-(+)-calystegine B_2 by Cac^+ and Cac^- *R. meliloti* 41. Wild-type (Cac^+) and calystegine catabolism deficient (Cac^-) *R. meliloti* were cultured with calystegine B_2 and analogs as the sole carbon and nitrogen source. Calystegine catabolism was assessed as disappearance from the medium after 48 h. C: control without bacteria.

In previous studies[20] both a crude alkaloid extract from *C. arvensis* and purified, natural calystegines A and B (a mixture of the isomers B_1 and B_2) from *C. sepium* were shown to be strong inhibitors of almond β-glucosidase and *Aspergillus niger* α-galactosidase. However, their activity and specificity as glycosidase inhibitors varies with structure, the more highly hydroxylated calystegine B isomers being 1 or 2 orders of magnitude more potent than the calystegine A isomers.[20] The ability of calystegines to interfere with glycosidase action suggests stereochemical similarities between these inhibitors and the normal glucosidase or galactoside substrate. The natural enantiomer, (+)-calystegine B_2, inhibited both enzymes, with K_i values of 4 μM for β-glucosidase and 7 μM for α-galactosidase (Figure 3). These values are similar to those obtained for the mixture of calystegines B[20] and for calystegine B_2 isolated from *Morus* species[10,11] and are comparable with the potent inhibitory activity of the polyhydroxyindolizidine alkaloids swainsonine and castanospermine toward α-mannosidase and α- and β-glucosidases, respectively.[22] In contrast, no inhibitory effect was seen against β-glucosidase and α-galactosidase with the unnatural enantiomer, (−)-calystegine B_2, even at con-

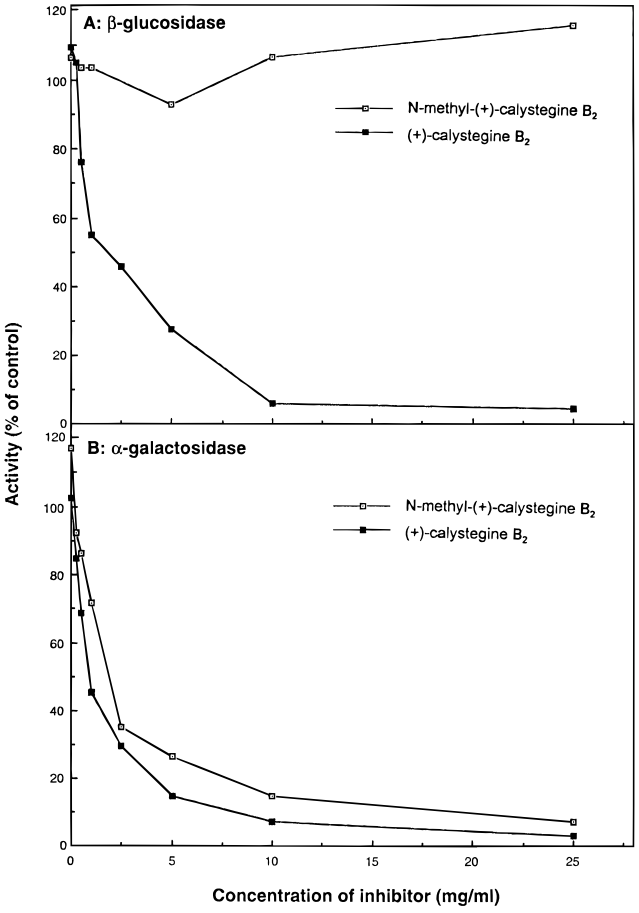

Figure 3. Glycosidase inhibitory activities of (+)-calystegine B_2 and its *N*-methyl derivative. No inhibition was detected with (−)-calystegine B_2 against α-glucosidase and *β*-galactosidase at 0.5 and 1.0 mg/mL (data not shown).

centrations as high as 0.5 and 1.0 mg/mL (data not shown). Surprisingly, *N*-methyl-(+)-calystegine B_2 inhibited α-galactosidase to a similar extent as (+)-B_2 but failed to have any activity toward *β*-glucosidase (Figure 3). Thus, methylation of the nitrogen to give a tropane analog corresponding to the naturally occurring nortropane altered the specificity of the inhibitor.

Glycosidase inhibitors are of interest as potential antitumor and antiviral agents.[22] A mixture of calystegines A and B from *C. sepium* was therefore tested for HIV inhibition (Diagen, Düsseldorf), but no activity was detected.

Numerous biological processes are dependent upon glycosidases for proper cellular function, suggesting that calystegines could act as toxins and inhibit the growth of rhizosphere bacteria unable to degrade them. We thus tested their effects on the growth of a Cac^+ strain (*R. meliloti* 41), on two Cac^- *Rhizobium* strains (*R. meliloti* GMI13 and RCR 2011), and on two Cac^- *Agrobacterium* strains (*A. tumefaciens* C58C1 and *A. rhizogenes* HRI). As expected from previous work, the natural (+)-calystegine B_2 was catabolized and stimulated the growth only of the wild Cac^+ *R. meliloti* strain but neither (+)- nor (−)-enantiomer inhibited the growth of either Cac^- or Cac^+ bacteria.

In contrast, calystegines inhibited *Medicago sativa* seed germination and root elongation. Synthetic (+)-calystegine B_2, the natural enantiomer, inhibited germination to 50% at 5 mM, while the unnatural (−)-enantiomer was ineffective at concentrations up to 10 mM (Figure 4). Regulation of root growth was studied using *M. sativa* transformed by *A. rhizogenes*. Root elongation rates were reduced by 37% and 46% after 19 h with 5 and 10 mM (+)-calystegine B_2, respectively, and by 12% and 40% after 43 h. Similar effects were observed on lateral root production and elongation (data not shown). When roots were incubated with 5 and 10 mM (−)-calystegine B_2, an inhibitory effect (20% and 25%) was observed during the first 19 h, and at 43 h elongation increased 8% and 18% at the two concentrations tested. The allelopathic effects of (+)-calystegine B_2 were confirmed with calystegine extracts from *A. belladonna* and *C. sepium*.

Plants and rhizosphere bacteria clearly engage in dedicated relationships,[23] and plant secondary metabolites, which are exuded in large amounts[24,25] have long been thought to play a nutritional role in the specificity of these interactions.[26−28] We have examined calystegines as possible nutritional mediators of specific as-

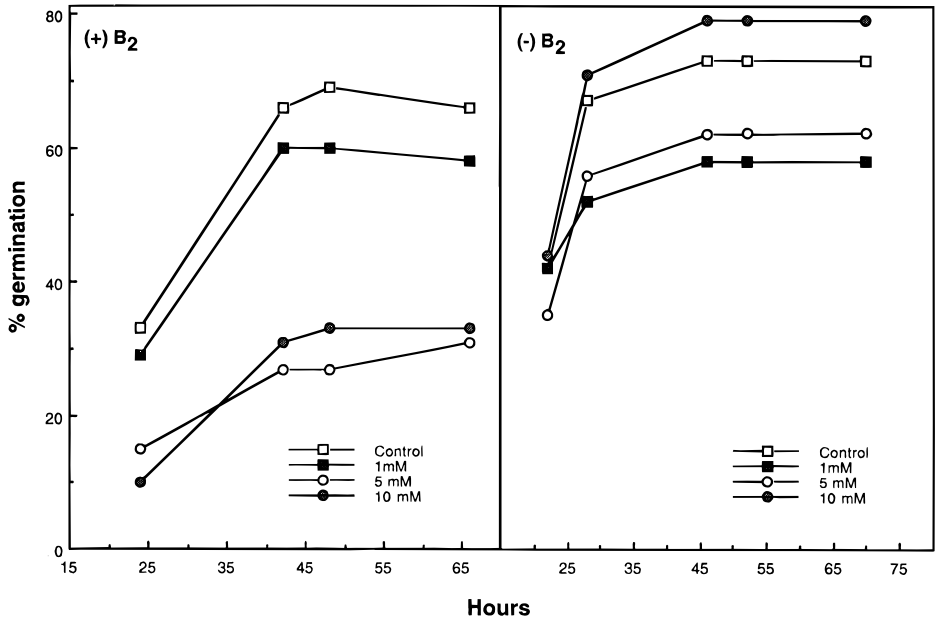

Figure 4. Effect of (+)- and (−)-calystegines B_2 on the germination of *M. sativa* seeds.

sociations in the rhizosphere. Over 20% of the bacteria isolated from the rhizospheres of plants that synthesize calystegines (Cas^+) were able to catabolize calystegines (Cac^+). In contrast, the Cac^+ phenotype was not found in bacteria from the rhizospheres of plants (Cas^-) that were unable to synthesize calystegines, indicating that the Cac^+ phenotype confers a selective advantage in the rhizosphere of Cas^+ plants.

Adaptation of rhizosphere bacteria to a particular host could be facilitated by the sharing of a catabolic plasmid, *e.g.*, pRme41a.[17] However, since DNA sequences homologous to the calystegine catabolism region of pRme41a were not detected in three wild (Cac^+) isolates, we conclude that the Cac^+ phenotype is not simply due to conjugative transfer of the *Rhizobium* catabolic plasmid or a recent derivative; rather, it results from highly divergent or independent evolution of basic catabolic functions. It must therefore be presumed that *cac* genes arose through coevolution between soil bacteria and calystegine-producing hosts. Among these wild, Cac^+ bacteria, a *P. fluorescens* was recovered, which would be a candidate for plant growth-promoting activity through siderophore or antibiotic production,[29] reinforcing the idea that a plant host could use calystegines to selectively promote certain beneficial soil bacteria.

Because they are glycosidase inhibitors, calystegines may, as reported for other polyhydroxyalkaloids,[30−32] serve as protective constituents, having toxic effects on competing plants, herbivores, and pathogens. In this scenario, their degradation by genetically adapted soil bacteria would not only provide nutrition but also serve as a detoxification mechanism. Calystegines did not have antimicrobial activities against two noncatabolic *Rhizobium* (GMI13 and 2011) and two *Agrobacterium* (C58C1 and HRi) strains that were tested, but the possibility exists that they interfere with specific functions requiring glycosidase activities, *e.g.*, the activation of glycosylated flavones, necessary for the recognition of a legume host by *Rhizobium*.

Calystegines inhibited both seed germination and root elongation in *M. sativa*. Inhibition of tomato seed germination was also observed (results not shown). Similar results have been shown for the polyhydroxy indolizidine alkaloid glucosidase inhibitor, castanospermine.[33] Allelopathic secondary metabolites in root exudates are thought to be important in the establishment and maintenance of plant communities.[34] It is possible that plants competing with Cas^+ species could associate with Cac^+ soil bacteria as a defense against calystegines. It remains to be shown, however, that calystegines have allelopathic properties under natural conditions. Such interactions are known to be target dependent, so that species other than *M. sativa* may be more susceptible to growth inhibition by these alkaloids, and since secondary metabolites are probably released at continuous low levels, rather than in the single dose, the bioassay results are only indicative of allelopathic potential. Furthermore, because calystegines are present in the leaves of some species, with levels being highest in the young growth, their ability to modulate plant/insect and insect/predator interactions has also been postulated.[7,8]

The natural calystegines from *C. sepium*, A_3 (**1**), B_1 (**4**), and B_2 (**5**), have a structural feature in common with respect to the hydroxyl substituents on carbons C-1, C-2, and C-3.[2] All three calystegines are substrates for bacterial catabolism, showing that additional hydroxylation of calystegine A_3 at C-6 to form calystegine B_1, or at C-4 to form calystegine B_2, does not modify catabolism. The recently detected calystegines A_5 (**2**), B_3 (**6**), and C_1 (**7**) have a different hydroxylation pattern,[9] but the action of bacteria upon these has not been tested. Although the information is incomplete, bacterial catabolism among the natural calystegines is presumably the rule. The complexity of the cac region in *R. meliloti*[18] is probably a reflection not only of the difficulty of cleaving the nortropane bicyclic ring system but also of the diversity in calystegine structures.

Glycosidase inhibition has been examined for the seven known natural calystegines,[9,20] showing that an equatorial OH group on C-2 is essential for the inhibition of β-glucosidase and the α- and β-galactosidases, that alteration of the stereochemistry of this hydroxyl group from an equatorial to an axial configuration decreased inhibitory activity, and that hydroxylation at C-6 influenced specificity. In addition, calystegine C_1, the most hydroxylated of this class of alkaloids with five −OH groups, was also the most potent inhibitor toward β-glucosidases. Thus, as reported for other polyhydroxylated alkaloids,[32,35] glycosidase inhibition by natural calystegines is modulated by the number, position, and stereochemistry of the hydroxyl groups.

The chiral synthesis of (+)- and (−)-calystegine B_2 permitted a comparison of the biological properties of the two enantiomers to be made. As expected, biological activity was dependent on the chirality. Only (+)-calystegine B_2 was catabolized by *R. meliloti* 41 and showed activity as a glycosidase inhibitor and an allelopathic compound. Thus, the (−)-enantiomer probably is not recognized by the bacterial catabolic enzymes and by the eucaryotic β-glucosidase and α-galactosidase. However, the (−)-calystegine B_2 enantiomer did exhibit slight stimulation of root elongation over the total time period of the assay, even though root length was initially decreased relative to controls, suggesting that the alkaloid might affect cell wall glycosidases involved in growth to some extent. This is surprising, since one would not expect, and there is no evidence to suggest, that the enantiomer occurs naturally.

Methylation of the nitrogen atom in calystegine B_2 abolished bacterial catabolism and suppressed the inhibition of β-glucosidase; however, *N*-methylation did not interfere with α-galactosidase inhibition, presumably due to differences in the active sites of the two glycosidases. An absence of α-galactosidase in humans is responsible for the lysosomal storage disorder, Fabry's disease, which results in the accumulation of neutral glycolipids in many tissues due to incomplete glycoprotein processsing. As a potent inhibitor of the enzyme, *N*-methylcalystegine B_2 might serve to chemically induce a similar deficiency, providing a useful experimental model for the genetic disease. *N*-Alkylation alters glycosidase inhibition by the polyhydroxylated piperidine alkaloid deoxynojirimycin and by synthetic aminosugars,[36,37] probably through a conformational change in the alkylated molecules.[38] The large class of tropane alkaloids, typified by scopolamine, are *N*-methylated, as is their common biosynthetic precursor, tropinone. Therefore, in contrast to the unnatural enantiomers,

Table 1. Bacteria and Plasmids

		ref
R. meliloti		
RCR 2011	wild type, isolate from alfalfa, Cac$^-$	39
41	wild type, isolate from alfalfa, Cac$^+$	39
AK631	nonmucoid derivative of 41	40
GMI13	AK631 cured of pRme41a, Cac$^-$	17
Pseudomonas		
A11, D1	Cac$^+$ wild type, isolates from rhizosphere of *C. sepium*	this work
plasmids		
pRme41a	Cryptic plasmid from *R. meliloti* 41, Cac$^+$, Tra$^+$	4
pGMI4103	RP4 derivative, carrying a fragment of pRme41 cloned in the Hind III site, Tc^r, Ap^r	P. Boistard

N-methylated derivatives might result from a simple mutation, *e.g.*, loss of function, during the course of calystegine biosynthesis. Thus, it appears likely that *N*-methylated polyhydroxy alkaloids corresponding to the calystegines will eventually be isolated and identified.

Calystegines appear to have multiple functions, acting as stimulators of growth of plant-associated microbes as well as probable inhibitors of competing plants and perhaps soil microbes, although no evidence is presently available to support this latter possibility. The presence of *cac* genes in *R. meliloti* would be nutritionally advantageous to the bacterium in the rhizosphere of Cas$^+$ plants, such as the bindweeds or morning glories, *C. sepium* and *C. arvensis*. In addition, a Cac$^+$ *Rhizobium* species could enable alfalfa, the germination and growth of which is inhibited by the calystegines, to compete against such Cas$^+$ plants. Since morning glories are important weeds, it would be useful to better understand the phytochemical basis for their ecological success and thereby attempt to manipulate the rhizosphere environment to suppress their growth or to stimulate the competitiveness of desirable species.

Experimental Section

General Experimental Procedures. GC−MS analyses of the calystegine TMSi derivatives, prepared by treatment with MSTFA in pyridine at 60 °C for 1 h, were performed on a Hewlett-Packard 5890 Series II instrument equipped with a 5971 mass-selective detector operating at 70 eV, on-column injector, and a 60 m × 0.32 mm i.d. SE-30 fused Si column. The column was temperature programmed from 120 to 300 °C at 10°/min.

The (+)- and (−)-enantiomers of calystegine B_2 were synthesized as described.[12,14] *N*-methyl-(+)-calystegine B_2 was prepared by O. Duclos, A. Duréault, and J.-C. Depezay (unpublished results). The glycosidases and the *p*-nitrophenyl glycoside substrates were purchased from Sigma.

Plant Material. *C. sepium* and *C. arvensis* were collected from wild populations in the vicinity of the Institut National de la Recherche Agronomique, Versailles, France.

Bacteria. Bacterial strains are described in Table 1. *Rhizobium* and *Pseudomonas* were grown in TY medium (0.5% tryptone, 0.3% yeast, 2 mM $CaCl_2$), and *A. tumefaciens* was cultured as previously described.[42] Minimal medium was MS for *Rhizobium*.[43] Minimal medium for growth tests was supplemented with calystegines as the sole carbon and nitrogen source. For toxicity tests, calystegines were added to complete medium. Bacterial growth was monitored as turbidity at 650 nm.

(+)-Calystegine B_2 (5) and (−)-Calystegine B_2 (8). The trimethylsilylated synthetic (+)- and (−)-calystegine B_2 enantiomers had the same retention times, t_R 16.25 min, and fragmentation pattern, EIMS *m/z* $[M]^+$ 463 (0.1), 448 (1), 373 (4), 284 (5), 259 (11), 244 (9), 229 (6), 217 (100), and 156 (6), on GC−MS, identical to those of the tetrakis(trimethylsilyl) derivative of natural-(+)-calystegine B_2.[5]

***N*-Methyl-(+)-calystegine B_2 (9).** The trimethylsilylated alkaloid gave a single peak on GC−MS, t_R 16.53 min. The fragmentation pattern, EIMS *m/z* $[M]^+$ 477 (6), 462 (6), 388 (22), 273 (100), 258 (22), 184 (13), 171 (72), 170 (52), and 147 (20), established the derivative as tetrakis(trimethylsilyl)-*N*-methyl-(+)-calystegine B_2.

Isolation of Cac$^+$ Bacteria from the Rhizospheres of *C. sepium* and *C. arvensis*. Root fragments and adhering soil particles from plants growing in the wild were stirred in 50 mL of sterile water for 30 min. Samples (200 mL) from serial dilutions (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) were spread on medium containing 5% peptone, 2.5% yeast extract, and 1% glucose, supplemented with two fungicides, nystatin (50 mg/mL) and metalaxyl (20 mg/mL), and incubated at 28 °C for 2 days. Bacterial colonies were picked at random and purified twice on the same medium.

Hybridization Experiments. Isolation of total DNA from *R. meliloti* and wild Cac$^+$ bacteria was accomplished according to published methods.[44] Plasmid Rme41a was provided by C. Rosenberg (INRA, Toulouse). DNA was cut with HindIII. Molecular hybridization was performed with commercial materials for nick translation and blotting (Amersham). Hybridization and washing was in 2 × SSC at 65 °C. The probe covering the cac region of pRme41a (pGMI4103) was provided by T. Huguet, INRA, Toulouse.

Catabolic Tests, Glycosidase Assays, and Allelopathic Tests. For catabolic tests, *R. meliloti* 2011 (OD_{650} 0.5) was incubated at 28 °C for 48 h with agitation in 200 mL of MS minimal medium containing 50 mg of calystegines as the sole carbon and nitrogen source. Calystegine disappearance from the medium was measured using high voltage paper electrophoresis and silver staining.[17] Glycosidase assays were performed as previously described.[20]

Allelopathic effects were measured in germination and root elongation tests, which were performed using a mixture of natural calystegines and then repeated using the synthetic calystegines. Results of the latter assays are given here. Seeds of *Medicago sativa* var. 'Europa' were surface-sterilized and germinated in 96-well microtiter dishes in a growth chamber at 24 °C; each well contained 10 mL of water supplemented with the (+)- or (−)-calystegines B_2 (1.5 or 10 mM). Root elongation was measured using transformed root cultures of *M. sativa*, established as described for other species.[45,46] Transformed roots were grown as organ clones in liquid MS medium[47] with the total nitrogen reduced to one fifth. To test the effects of calystegines on root elongation, 20 apical, 1 cm segments were

excised from log phase cultures of a root clone grown in liquid medium and placed on 5 × 8 cm glass plates that had been covered with a 2.5 mL film of solid medium, containing a calystegine at 0, 1, 5, or 10 mM. These glass plates were incubated on a water/agar medium in Petri dishes sealed with a PVC film. Root elongation was measured by marking the advancement of the root tip on the underside of the Petri dish. The statistical validity of the results was verified using the unpaired t test.

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References and Notes

(1) These compounds were originally named as "calystegins", but the term "calystegines" has subsequently been adopted by certain research groups. Because the former name was previously assigned to a different class of compounds (Chu, J.-H.; Chian, Y.-H. *Chem. Zentralblatt.* **1960**, *131*, 1859−1860), and in order to conform with the convention used for naming other polyhydroxy alkaloids, the latter term is now preferred.
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