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Nitrogenous Ovipositional Deterrents in the Leaves of Sweet Pepper (*Capsicum annuum*) at the Mature Stage against the Leafminer, *Liriomyza trifolii* (Burgess)

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Mature leaves of the sweet pepper, *Capsicum annuum*, exhibited resistance against the American serpentine leafminer, *Liriomyza trifolii* (Burgess), Agromyzidae. Based on bioassay-guided fractionation, three compounds, namely 4-aminobutanoic acid, (2*S*,4*R*)-4-hydroxy-1-methyl-2-pyrrolidine carboxylic acid and 4-amino-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone, were isolated from the leaves of sweet pepper. These compounds had significant oviposition deterrence towards adult flies of *L. trifolii* from laying their eggs on host plant leaves treated at 3.70, 16.60 and 6.45 $\mu g/cm^2$, respectively.

Key words: *Liriomyza trifolii*; ovipositional deterrent; *Capsicum annuum*; Solanaceae; nitrogenous compound

Liriomyza trifolii (Burgess) is a major leafminer pest to a variety of vegetables, and ornamental plants, including tomato, cucumber, lettuce, melon,[1] celery, and chrysanthemums.[2] The fly, which originated in North America,[3] has spread worldwide, was first found in Japan, Shizuoka and Aichi prefectures in 1990,[4] and has established itself nationwide as a significant pest. The female leafminer punctures the leaves of plants for feeding and oviposition by using her ovipositor.[5] The larval stage of the flies feed on the inner tissues of the host leaves, and at a high fly density, this feeding severely reduces the yield or even kills the plants.[5] One of the difficulties in controlling this species is its ability to quickly develop resistance to insecticides,[6–8] and thus studies on alternative methods of pest control are underway.[5]

While conducting a survey of host selection for *L. trifolii* in a greenhouse, we observed that the leaves of sweet pepper (*Capsicum annuum*) at a mature stage were rarely attacked by this insect.[9] On the other hand, this species attacks the young leaves of sweet pepper. We have previously reported luteolin 7-*O*-β-D-apiofuranosyl-(1 $\rightarrow$ 2)-β-D-glucopyranoside[9] and phytol {(2*E*)-3,7,11,15-tetramethyl-2-hexadecen-1-ol}[10] from the methanol extract of mature *C. annuum* leaves as ovipositional deterrents against this insect species. In the course of isolating these deterrents, we reported the presence of activity in the aqueous fraction.[9] We report here the components of the aqueous fraction of sweet pepper leaves which were responsible for the resistance to *L. trifolii*.

Results and Discussion

Ovipositional response of L. trifolii on kidney bean leaves

The active methanol extract was partitioned between hexane, diethyl ether, ethyl acetate, water-saturated butanol and water layers. The water layer was then separated into its supernatant (aqueous fraction) and precipitate by centrifugation. Treatment of the kidney bean leaf with the aqueous fraction resulted in a significant reduction in the number of leaf punctures (Table 1). This result clearly indicated the presence of active constituents in the aqueous fraction. The aqueous fraction (3.7 g, 100 g of fresh leaf equivalent) was applied to ODS MPLC and eluted with a decreasing polarity of water/MeOH mixtures to give the H_2O fr., 20% MeOH/H_2O fr., 40% MeOH/H_2O fr. and MeOH fr. The H_2O fr. decreased the number of ovipositional marks (9.10 marks/cm^2 $\pm$ 1.23) compared to those of the control leaves (32.00 ± 3.04), while the other fractions did not result in any significant decline in the number of oviposition marks. The active H_2O fr. was then fractionated in to the basic, neutral and acidic frs. by ion-exchange chromatography. Kidney bean leaves treated with the basic fraction exhibited significantly fewer oviposition marks (9.38 ± 0.38), while the neutral and acidic frs. showed no significant decline in the numbers of oviposition marks. Separation of the basic fraction by reverse-phase HPLC afforded three fractions

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Table 1. Number of Ovipositional Marks Made by *L. trifolii* on Kidney Bean Leaves Treated with the Test Sample Solutions and the Control

Treatment	Ovipositional marks/cm^2
Aqueous fraction	10.33 ± 1.65^a
Water fraction	9.10 ± 1.23^a
20% MeOH/H_2O fraction	28.00 ± 3.61^b
40% MeOH/H_2O fraction	30.00 ± 3.20^b
MeOH fraction	29.00 ± 0.76^b
Basic fraction	9.38 ± 0.38^a
Neutral fraction	20.20 ± 0.65^b
Acidic fraction	29.20 ± 2.80^b
Fraction 1	8.40 ± 1.22^a
Fraction 2	10.60 ± 0.31^a
Fraction 3	28.00 ± 0.41^b
1	9.37 ± 0.33^a
2	8.30 ± 1.04^a
3	10.20 ± 1.43^a
1 + **2** + **3**	8.40 ± 0.33^a
Control	32.00 ± 3.04^b

Each value is expressed as the mean ± S.E. Data were analyzed by ANOVA, followed by the Tukey-Kramer HSD test for multiple comparisons. Values with different superscript letters indicate significance difference, $p < 0.05$.

(Frs. 1–3). Fr. 1 (8.4 ± 1.22) and Fr. 2 (10.60 ± 0.31) exhibited ovipositional deterrence, while Fr. 3 failed to show any such activity. Finally, compounds **1**–**3** were isolated by HPLC from Frs. 1 and 2. *L. trifolii* were deterred from ovipositing on kidney bean leaves treated with **1**–**3**, the numbers of ovipositional marks being 9.37 ± 0.33 with **1**, 8.30 ± 1.04 with **2** and 10.20 ± 1.43 with **3**. The calculated contents of **1**–**3** were 20 μg/g (3.70 μg/cm^2), 84 μg/g (16.6 μg/cm^2) and 32 μg/g (6.45 μg/cm^2), respectively, in fresh *C. annuum* leaves at the mature stage.

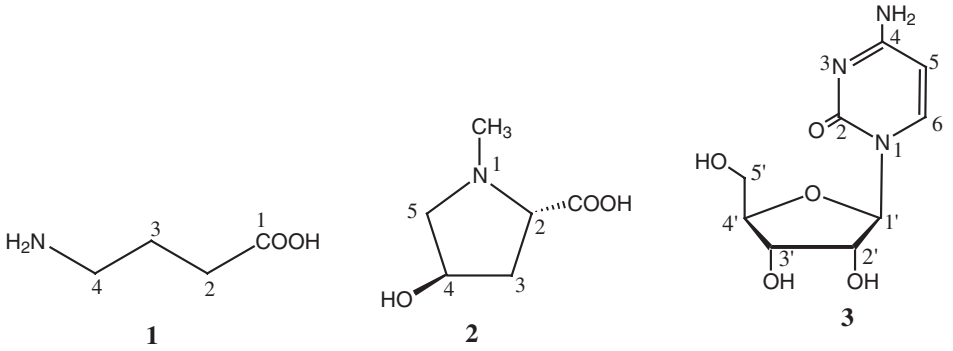
Structural determination

Compound **1** was obtained as an amorphous solid. The positive-ion APCI-MS data gave pseudomolecular ions at m/z 104 $[M + H]^+$, 122 $[M + H + H_2O]^+$ and 145 $[M + H + H_2O + Na]^+$. The APCI-MS and ^{1}H- and ^{13}C-NMR data enabled the molecular formula of $C_4H_9NO_2$ to be deduced. The ^{1}H-NMR spectrum of **1** indicated vicinal coupling between a proton signal at 1.88 (2H) ppm with signals at 2.28 (2H) and 2.98 (2H) ppm. The HH-COSY data demonstrated the correlation of protons at 1.88 ppm with those at 2.28 ppm and 2.98 ppm and vice versa, indicating the presence of methylene protons at 1.88 ppm sandwiched between two pairs of methylene protons to give the $-CH_2-CH_2-CH_2-$ skeleton. ^{13}C-NMR and DEPT spectra indicated the compound to have three methylene carbons and a carboxyl carbon. Based on this evidence **1** was elucidated to be 4-aminobutanoic acid (Fig. 1). The chemical data of **1** were compared with those of a commercial sample of 4-aminobutanoic acid and found to be in good agreement.

4-Aminobutanoic acid, commonly known as γ-aminobutyric acid (GABA) is an intermediate in the normal catabolism of glutamic acid in organisms, its accumulation in plants in response to biotic and abiotic stresses being mediated *via* the activation of glutamate decarboxylase.[11] Various uncooked foods have been reported to contain significant amounts of γ-aminobutyric acid.[12] Brown rice, sprouting cereals and spinach are good sources of plant-derived GABA.[12] Elevated levels of endogenous GABA in genetically engineered tobacco deter feeding by tobacco budworm larvae and infestation by the northern root-knot nematode.[13]

Compound **2** had the molecular formula of $C_6H_{11}NO_3$ as deduced from the APCI-MS data measured in the positive-ion mode at m/z 146 $[M + H]^+$, 168 $[M + Na]^+$ and 184 $[M + K]^+$, and from ^{1}H- and ^{13}C-NMR data. The ^{13}C-NMR and DEPT spectra showed signals for 6 carbon atoms corresponding to one methyl, two methylenes, two methines and a carboxyl carbon. The ^{1}H-NMR spectrum showed a singlet at 2.87 ppm (3H), while geminally coupled aliphatic protons appeared at 2.07 and 2.30 ppm as well as at 3.01 and 3.77 ppm, indicating that the compound had an *N*-methyl group and two aliphatic methylenes. The methine proton signals observed at 4.01 (1H) and 4.45 (1H) ppm were due to the attachment to nitrogen and oxygen, respectively based on their chemical shifts. Both protons at 4.01 and 4.45 ppm were coupled with aliphatic methylene protons at 2.07 and 2.30 ppm. Thus, the compound seemed to have a pyrrolidine skeleton, and its structure was elucidated to be 4-hydroxy-*N*-methylproline. Compound **2** was then prepared from L-4-hydroxyproline by reductive amination with formaldehyde,[14] its NMR data being in good agreement with those of **2**. The absolute configuration of the compound was determined to be 2*S*,4*R* by comparing its specific degree of rotation, $[\alpha]_D^{22} = -44.1^\circ$ ($c = 1.2$, H_2O), with that of the prepared compound. Based on the foregoing chemical data, **2** was identified as (2*S*,4*R*)-4-hydroxy-1-methyl-2-pyrrolidine carboxylic acid. 4-Hydroxy-*N*-methylproline is an unusual amino acid which has never before been found from the family Solanaceae and has been reported in species such as the red alga, *Chondria coerulescens*, and higher plants (*Croton gobouga* and *Afrormosia elata*).[15] Moreover, isolation of the compound from *Toddalia asiatica* and its oviposition stimulatory activity for the swallowtail butterfly (*Papilio polytes*) has been reported.[16] It is important to note that this compound has had contrary effects towards the butterfly and *L. trifolii* flies. The occurrence of **2** in leaves of the *Copaifera* species and its strong inhibition against the larval development of the bruchid beetle (*Callosobruchus maculatus*) and its significant feeding deterrence of the leaf-feeding lepidopteran, *Spodoptera littoralis*, have been reported.[17]

Compound **3** was isolated as an amorphous solid, its APCI-MS data showing pseudomolecular ions at m/z 244 $[M + H]^+$, 262 $[M + H + H_2O]^+$ and 282 $[M + K]^+$ in the positive-ion mode and at m/z 242 $[M - H]^-$ in the negative-ion mode, indicating the compound to



1 **2** **3**

Fig. 1. Structures of Active Compounds **1**–**3**.
4-Aminobutanoic acid (**1**), (2*S*,4*R*)-4-hydroxy-1-methyl-2-pyrrolidine carboxylic acid (**2**) and 4-amino-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (**3**).

have a molecular weight of 243. The ^{1}H-NMR spectrum showed eight protons, and the ^{13}C-NMR and DEPT spectral data exhibited nine carbons (one methylene, six methines and two quaternary carbons). Judging from the chemical shifts, the compound had a pentose moiety ($C_5H_9O_4$) and aromatic moiety constituting two methines, a quaternary carbon and a carbonyl carbon. As the molecular weight of the compound was an odd number, 243, the structure consisted of the remaining three nitrogen atoms and two protons. The ^{1}H-NMR spectrum exhibited six sugar proton signals in the region of 3.6–5.8 ppm, including one anomeric proton signal at 5.71 ppm (*br-s*) and a proton signal at 3.62 ppm (*d*, $J = 12.4$ Hz) which was geminally coupled with that of 3.74 ppm (*d*, $J = 12.8$ Hz), indicating oxygenated CH_2 protons. The H–H COSY spectral proton signals at 3.62 and 3.74 ppm showed a cross peak, indicating geminal coupling and a cross peak with a signal at 3.94 ppm which demonstrated the connectivity of O–CH_2–CH– in the sugar unit. Similarly, H–H cross peaks were observed between the signals at 4.01 and 4.12 ppm, and 4.12 and 5.71 ppm. This evidence confirmed the presence of a five-membered sugar ring, a ribofuranosyl moiety, in the structure. Furthermore, two vicinally coupled olefinic protons appeared at 5.86 (1H, *d*, $J = 5.2$ Hz, H-5) and 7.65 (1H, *d*, $J = 7.2$ Hz, H-6) ppm which was also confirmed by H–H COSY. The H-6 proton was significantly shifted downfield, indicating the conjugation of the double bond with an electron-withdrawing group. The anomeric carbon was shifted upfield (90.9 ppm) indicating the ribofuranosyl moiety, and the main skeleton was connected to a nitrogen atom. It is reasonable from this information that this compound had the pyrimidinone skeleton. Based on the ^{1}H-, ^{13}C-NMR and APCI-MS data, the molecular formula of $C_9H_{13}N_3O_5$ was determined and the compound was characterized to be cytidine. By comparing its specific degree of rotation, $[\alpha]_D^{22} = +26.9^\circ$ ($c = 1.2$, H_2O), with that of an authentic sample, it was identified as 4-amino-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (cytidine). Cytidine is a non-toxic endogenous nucleoside that is formed when cytosine is attached to a ribose ring (a ribofuranose) *via* a β-N_1-glycosidic bond; it is one of the important components of DNA and thus is present in almost all organisms and is an abundant compound in nature.[18] We report here, for the first time its unique use by the plant against pests. Additionally, its antidepressant-like effects in the forced swim test in rats are known.[19]

The ovipositional resistance of *C. annuum* leaves against *L. trifolii* is attributed to the soluble constituents in the water layer {4-aminobutanoic acid, (2*S*,4*R*)-4-hydroxy-*N*-methylproline, cytidine and luteolin 7-*O*-β-D-apiofuranosyl-(1 $\rightarrow$ 2)-β-D-glucopyranoside} and the hexane layer (phytol). Cucurbitane triterpenoids, such as momordicine I, 7,23-dihydroxy-3-*O*-malonylcucurbita-5,24-dien-19-al, momordicine II and momordicine IV isolated from *Momordica charantia* have shown strong ovipositional deterrent activity against *L. trifolii*.[20,21] We have reported various classes of compounds, namely terpenoids, flavonoid glycosides and basic compounds, as strong ovipositional detterents against *L. trifolii* pests.

Several nitrogen-containing compounds have been reported to have antifeedant and insecticidal effects against various insects.[22,23] For instance, ryanoid diterpenes have antifeedant activity against larvae of the cotton leafworm (*Spodoptera littoralis*) and Colorado potato beetle (*Leptinotarsa decemlineata*).[22] Sesquiterpene alkaloids isolated from *Celastrus angulatus* (Celastraceae) have shown strong antifeeding action against several insect species.[23] Accordingly, although **1**–**3** are not alkaloids, the presence of nitrogenous functional groups in their structures might play an important role in their ovipositional deterrence of *L. trifolii*. It is well known that various secondary metabolites are utilized as chemical defence by plants against attacking insects. It is quite interesting to note that 4-aminobutanoic acid and cytidine, which are the primary metabolites, are used by sweet pepper for defense against leafminers. Primary metabolites such as L-malic acid and isocitric acid have previously been isolated from finger millet (*Eleusine coracana*) and reported to have an antifeedant effect against the brown planthopper (*Nilaparvata lugens*).[24] There were no synergistic effects on the ovipositional

deterrence when the foregoing three basic compounds (**1–3**) were combined in their natural abundance and bioassayed (8.40 ± 0.33 marks/cm^2), indicating that treatment of these compounds at a dose of 1 g of fresh leaf equivalent/ml would be close to the constant-value response (no synergism). As there is no report on the toxic effects of these compounds, they may be utilized as a safe and environmentally compatible control method against *L. trifolii*. We have indicated in the previous report that as the plant matured the concentration of luteolin 7-*O*-β-D-apiofuranosyl-(1 $\rightarrow$ 2)-β-D-glucopyranoside increased.[9] A study based on the dose response of **1–3** and phytol at different leaf stages is important as the resistance of the plant against the leafminer increases as it matures.[9]

Experimental

Instruments. HPLC for the isolation and analysis of the compounds was carried out with a reversed-phase column (Shiseido capcell pak C_{18} AQ, 250 mm $\times$ 10 mm i.d.), a Hitachi L-6000 pump equipped with an SPD-10 AVP Shimadzu UV–VIS detector, a CTO-10AC VP Shimadzu column oven and a Hitachi D-2500 Chromato Integrator (chart speed of 2.5 mm/min). The mobile phase was water at a flow rate of 2 ml/min for all compounds. MPLC (Yamazen pump 600A) was used for fractionation.

NMR data were recorded by a JEOL JNM-L400 spectrometer in D_2O and CD_3OD with TMS as an internal standard. Chemical shifts are represented by the δ unit, and the multiplicity of signals is abbreviated as singlet (*s*), doublet (*d*), triplet (*t*), quartet (*q*) and multiplet (*m*). Coupling constants (*J*) are given in Hz. LCMS data were measured by a Shimadzu LC-MS 2010 liquid chromatograph-mass spectrometer with APCI mode.

Insect and plant. Stock colonies of *L. trifolii* were obtained from Kochi Prefecture Agricultural Center and successively reared by feeding 10–14-day-old seedlings of the kidney bean, *Phaseolus vulgaris*, at 27 ± 2 °C, a relative humidity of 60–70% with 16:8 (L:D) illumination. One-day-old females were collected randomly and used for the bioassay.

C. annuum var. angulosum were planted in a plastic tray (20 cm $\times$ 27 cm length, 1 cm in depth) containing nursery soil in a plant growth incubator at 27 ± 2 °C. After four weeks, the seedlings were transplanted into individual pots (6.6 cm height, 5.0 cm i.d.) containing nursery soil and grown in a greenhouse without any application of insecticides.

Bioassay. The same bioassay method was used as that previously reported.[9,10,20,21] A cut leaf of kidney bean was immediately dipped in the test solution prepared at 1 g of fresh leaf equivalent/ml of water for 30 s. After removing the solvent by air drying, the treated kidney bean leaf was put on a moistened filter paper at the bottom of a Petri dish (10 mm high, 90 mm i.d.) to maintain humidity. A control leaf was also treated in the same manner with only water. Five adult female flies at least 24 h old were put into a 50-ml screw-capped vial (28 mmϕ) and placed on the treated or control leaves by turning the screw vials upside down, allowing them to oviposit for 24 h at 27 °C under 16:8 (L:D) illumination. The number of leaf punctures made by the flies was counted after 24 h. Each test was replicated six times, and a statistical analysis was done by ANOVA and the Tukey-Kramer HSD test for multiple comparisons.

Extraction and isolation. Fresh leaves of *C. annuum* (1.2 kg) at the mature stage were extracted twice with 80% MeOH in water (3 l $\times$ 2) for 3 days in darkness at room temperature. The extracts were combined, filtered, and then the solvent was removed under reduced pressure to yield 89.0 g of a crude extract. The crude extract (89.0 g) was dissolved in water (1.5 l), and successively partitioned between hexane (1.5 l $\times$ 4, 16.80 g), diethyl ether (1.5 l $\times$ 4, 2.50 g), ethyl acetate (1.5 l $\times$ 4, 2.80 g) and water-saturated butanol (1.5 l $\times$ 4, 15.30 g) and water (50.30 g). Each layer was concentrated under vacuum. The water layer was then separated into the supernatant (aqueous fraction, 33.40 g) and precipitate (16.30 g) by centrifuging (3000 rpm at room temp. for 20 min). The aqueous fraction was then submitted to the bioassay. The aqueous fraction (3.70 g, 100 g of fresh leaf equivalent) was then applied to ODS MPLC and eluted with a decreasing polarity of a water/MeOH mixture to yield the water fraction (3.10 g), 20% MeOH fraction (0.20 g), 40% MeOH fraction (0.10 g) and MeOH fraction (0.08 g). The water fraction (3.10 g) was then subjected to cation-exchange chromatography (Dowex 50 $\times$ 8) and eluted with water (2-liter) to yield the neutral and acidic fraction (2.20 g). The column was then eluted with 2 *N* NH_3 (2-liter) and yielded the basic and amphoteric fraction (0.60 g). Subsequently, the neutral and acidic fraction (2.20 g) was applied to anion-exchange chromatography (Dowex 1 $\times$ 8) and eluted with water (2-liter) to afford the neutral fraction (0.80 g). The Dowex 1 $\times$ 8 column was then eluted with 20 *N* formic acid and yielded the acidic fraction (1.10 g). The basic and amphoteric fraction (0.60 g) was then fractionated by reverse-phase HPLC, eluting with water to yield three fractions, Fr. 1 (Rt = 0–9.10 min), Fr. 2 (Rt = 9.10–25.50 min), and Fr. 3 (Rt = 25.50–40.00 min). Further purification of Fr. 1 by HPLC under the foregoing conditions yielded **1** (Rt = 7.83 min) and **2** (Rt = 8.49 min), and Fr. 2 afforded **3** (Rt = 24.35 min). The fresh leaves of sweet pepper at the mature stage contained **1–3** at 20, 84 and 32 µg/g fresh leaf equivalent, respectively.

*4-Aminobutanoic acid (**1**).* Amorphous solid. ^{1}H-NMR (CD_3OD) δ: 1.88 (2H, *m*, H-3), 2.28 (2H, *t*, J = 6.8 Hz, H-2), 2.98 (2H, *t*, J = 6.8 Hz, H-4). ^{13}C-NMR

(CD_3OD): 24.8 *t* (C-3), 35.8 *t* (C-2), 40.6 *t* (C-4) and 181.4 *s* (COOH). LC-MS (APCI-positive) *m/z*: 104 $[M+H]^+$, 122 $[M+H+H_2O]^+$ and 145 $[M+H+H_2O+Na]^+$.

*(2S,4R)-4-Hydroxy-1-methyl-2-pyrrolidine carboxylic acid (**2**).* Amorphous solid. $[\alpha]_D^{22} = -44.1^\circ$ ($c = 1.2$, H_2O). ^{1}H-NMR (D_2O) δ: 2.07 (1H, *dt*, $J = 4.4$, 12.4 Hz, H-3a), 2.30 (1H, *dd*, $J = 7.6$, 12.4 Hz, H-3b), 2.87 (3H, *s*, N-*Me*), 3.01 (1H, *br-d*, $J = 13.2$ Hz, H-5a), 3.77 (1H, *dd*, $J = 4.4$, 13.2 Hz, H-5b), 4.01 (1H, *br-t*, $J = 7.6$ Hz, H-2), 4.45 (1H, *d*, $J = 2.0$ Hz, H-4); ^{13}C-NMR (D_2O) δ: 38.9 *t* (C-3), 43.8 *q* (N-*Me*), 63.3 *t* (C-5), 70.1 *d* (C-4), 70.7 *d* (C-2) and 173.4 *s* (COOH). LC-MS (APCI-positive) *m/z*: 146 $[M+H]^+$, 168 $[M+Na]^+$ and 184 $[M+K]^+$.

*Preparation of (2S,4R)-4-hydroxy-1-methyl-2-pyrrolidine carboxylic acid (**2**).* To a solution of L-hydroxyproline (1 g, 7.63 mmol) in water (5 ml) were added acetic acid (8.5 ml) and 37% aqueous formaldehyde (0.66 ml, 23.8 mmol).[14] The resulting mixture was stirred under H_2 atmosphere in the presence of PtO_2 (87 mg, 0.383 mmol) at 30 °C for 12 h. The catalyst was filtered away, and the resulting filtrate was evaporated under reduced pressure. The residue was diluted with acetone, and the precipitate was isolated by filtration to give (2*S*,4*R*)-4-hydroxy-1-methyl-2-pyrrolidine carboxylic acid (1.02 g, 92%) as a powder. $[\alpha]_D^{22} = -82.9^\circ$ ($c = 1.0$, H_2O). ^{1}H-NMR (D_2O) δ: 2.08 (1H, *dt*, $J = 4.0$, 14.0 Hz, H-3a), 2.32 (1H, *dd*, $J = 7.2$, 14.0 Hz, H-3b), 2.88 (3H, *s*, N-*Me*), 3.02 (1H, *br-d*, $J = 13.2$ Hz, H-5a), 3.78 (1H, *dd*, $J = 4.0$, 13.2 Hz, H-5b), 4.03 (1H, *br-t*, $J = 8.0$ Hz, H-2), 4.46 (1H, *d*, $J = 2.0$ Hz, H-4). ^{13}C-NMR (D_2O) δ: 38.9 *t* (C-3), 43.8 *q* (N-*Me*), 63.3 *t* (C-5), 70.1 *d* (C-4), 70.7 *d* (C-2) and 173.4 *s* (COOH).

*Cytidine or 4-amino-1-β-D-ribofuranosyl-2(1H)-pyrimidinone (**3**).* White powder. $[\alpha]_D^{22} = +26.9^\circ$ ($c = 1.2$, H_2O). ^{1}H-NMR (D_2O) δ: 3.62 (1H, *d*, $J = 12.4$ Hz, H-5′a), 3.74 (1H, *d*, $J = 12.8$ Hz, H-5′b), 3.94 (1H, *s*, H-4′), 4.01 (1H, *s*, H-3′), 4.12 (1H, *d*, $J = 3.2$ Hz, H-2′), 5.71 (1H, *br-s*, H-1′), 5.86 (1H, *d*, $J = 5.2$ Hz, H-5), 7.65 (1H, *d*, $J = 7.2$ Hz, H-6). ^{13}C-NMR (D_2O): 61.3 *t* (C-5′), 69.8 *d* (C-3′), 74.5 *d* (C-2′), 84.4 *d* (C-4′), 90.9 *d* (C-1′), 96.8 *d* (C-5), 142.2 *d* (C-6), 158.1 *s* (C-2) and 166.6 *s* (C-4). LC-MS (APCI-positive) *m/z*: 244 $[M+H]^+$, 262 $[M+H+H_2O]^+$ and 282 $[M+K]^+$. LC-MS (APCI-negative) *m/z*: 242 $[M-H]^-$.

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