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Studies on the Chemical Synthesis of Potential Antimetabolites. XXV.[1] Synthesis of (±)-Isowillardiine by Application of a Four-Component Condensation involving 2-Picolylamine 1-Oxide and 2-Picolyl Isocyanide 1-Oxide

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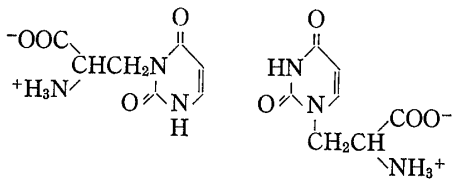
3-(2-Amino-2-carboxyethyl)uracil (racemic isowillardiine) was synthesized by the use of a four-component (aldehyde, amine, isocyanide, and carboxylic acid) condensation as the key reaction. Thus, 1-(2-picolyl 1-oxide)-3-(formylmethyl) uracil was allowed to react with 2-picolylamine 1-oxide and cyclohexyl isocyanide or 2-picolyl isocyanide 1-oxide in the presence of acetic acid in methanol to afford the corresponding condensation product, which after deblocking, gave (±)-isowillardiine in fair yield.

Keywords——isowillardiine; 2-picolylamine 1-oxide; 2-picolyl isocyanide 1-oxide; Ugi reaction; Four-component condensation; 2-picolyl 1-oxide protection

During the germination of pea seeds, various L-amino acids containing uracil, such as willardiine (2) and isowillardiine (1), are produced.[3,4] Among these, the structure of willardiine was established as 2 by unambiguous synthesis,[5] whereas to our knowledge, no reports have appeared dealing with the chemical synthesis of isowillardiine (1). Because of the current interest in the biosynthesis of isowillardiine,[6,7,8] we felt it appropriate to prepare 1 by chemical means. An outline of our approach is shown in Chart 1.



1 2

Some features of our approach to 1 are as follows. Since it is well documented[9] that the preparation of 3-alkyluracil from uracil by alkylation meets with only limited success, the sequence in Chart 1 starts with the easily accessible 1-(2-picolyl 1-oxide)uracil (3), from which the 2-picolyl 1-oxide[10] group is easily removable, as demonstrated previously.[11] The second point is that the latter part of our synthetic sequence involves "four-component (aldehyde,

1) *a*) Part XXIV: Y. Mizuno, T. Endo, Y. Inoue, H. Tampo, A. Takahashi, M. Iigo, A. Hoshi, and K. Kuretani, *Chem. Pharm. Bull.*, **28**, 1584 (1980); *b*) A part of this work was presented at the Symp. On the Chemistry of Natural Products at Sendai, Japan 1976 (Symposium Abstracts p. 53).
2) Location: *Kita-ku, Sapporo 060, Japan.*
3) F. Lambein and R. Van Parijis, *Biochem. Biophys. Res. Commun.*, **32**, 474 (1968).
4) E.G. Brown and B.S. Mangat, *Biochim. Biophys. Acta*, **177**, 427 (1969).
5) *a*) J.H. Dewar and G. Shaw, *J. Chem. Soc.*, **1962 583**; *b*) A. Kjaer, A. Knudsen, and P.O. Larsen, *Acta Chem. Scad.*, **15**, 1193 (1961); *c*) Yu. P. Shvachkin and M.T. Azarova, *Zh. Obshch. Khim.*, **34**, 407 (1964); *d*) A.P. Martinez and W.W. Lee, *J. Org. Chem.*, **30**, 317 (1965).
6) T.S. Ashworth, E.G. Brown, F.M. Roberts, *Biochem. J.*, **129**, 897 (1972).
7) F. Lambein and R. Van Parijis, *Arch. Int. Physiol. Biochim.* **78**, 595 (1970).
8) I. Murakoshi, F. Ikegami, N. Ookawa, T. Ariki, J. Haginiwa, Yu-Haey Kuo, and F. Lambein, *Phytochemistry*, **17**, 1571 (1976).
9) For example, B.R. Baker, and G.B. Chheda, *J. Pharm. Sci.*, **54**, 25 (1965).
10) This protecting group will occasionally be referred to as "op" hereafter.
11) Y. Mizuno, W. Limn, K. Tsuchida, and K. Ikeda, *J. Org. Chem.*, **37**, 39 (1972).

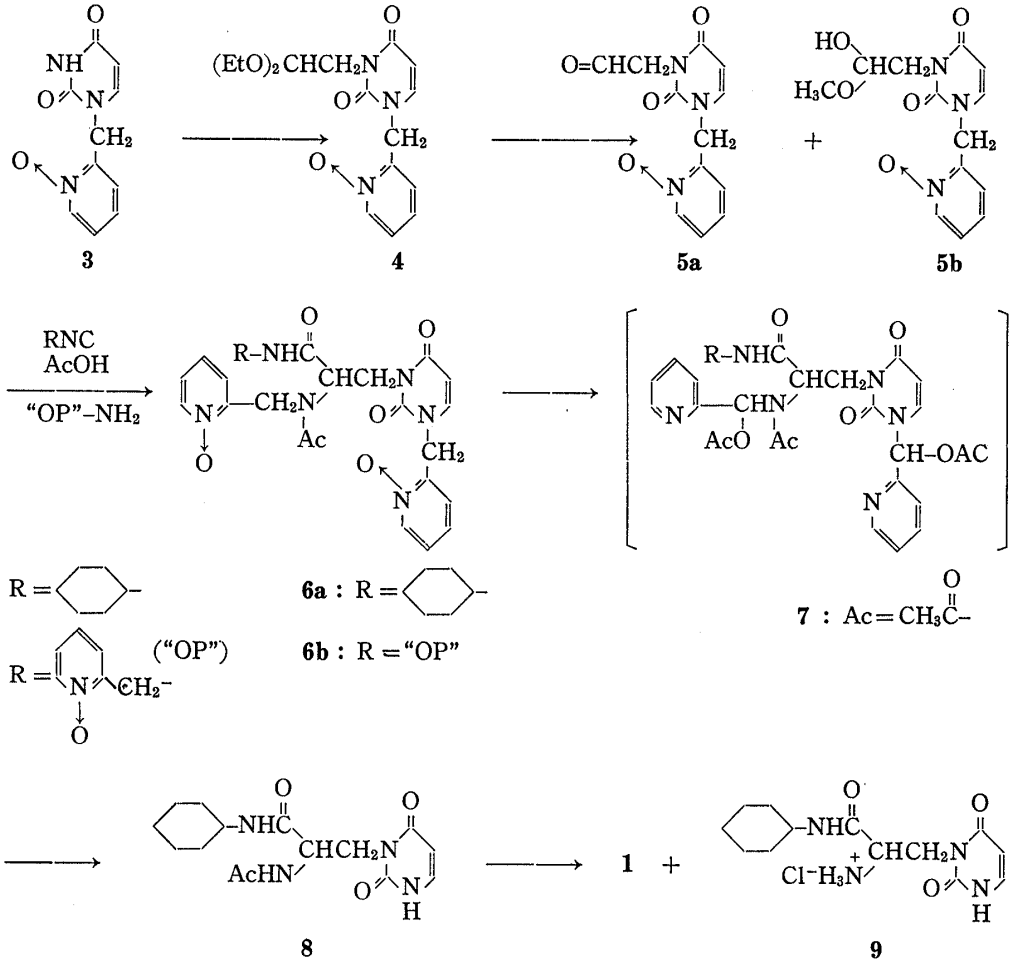


Chart 1

amine, isocyanide, and carboxylic acid)condensation"[12a] for the synthesis of the α-amino acid portion of isowillardiine (**1**), with 2-picolylamine 1-oxide (op-amine) and 2-picolyl isocyanide 1-oxide (op-isocyanide)[12b] as well as cyclohexyl isocyanide as amine and isocyanide components, respectively.

It should be noted that 2-picolylamine 1-oxide and 2-picolyl isocyanide 1-oxide, rather than usual alkyl amines (*i.e.*, benzyl amine) and alkyl isocyanide (*i.e.*, benzyl isocyanide), were used. Since the four-component condensation may give rise to *N*-substituted α-aminocarboxamides (for example, see, **6a** in Chart 1), the substituents on the two amino groups have to be removed in order to prepare unsubstituted α-amino acids. Thus, a benzyl group, for example, may be inappropriate, because the reductive de-benzylation also may reduce the uracil ring to the corresponding 5,6-dihydrouracil. As already mentioned, the 2-picolyl 1-oxide group should be easily removable by a procedure other than hydrogenation. In cases where op-protection was employed, the deblocking from three different kinds of nitrogen atoms (see **6b**, in Chart 1) could be effected simultaneously by a single procedure. Although 2-picolyl isocyanide 1-oxide is unknown in the literature, we have prepared the isocyanide in 71% yield by the reaction of (*N*-2-picolyl 1-oxide)formamide[or (*N*-picolyl 1-oxide)thioformamide],

12) *a*) P. Hoffman, G. Gokel, D. Marquarding, in "Isonitrile Chemistry," I. Ugi, Ed., Academic press, New York, 1971, pp. 19—35; *b*) Attempted syntheses of 2-picolyl isocyanide 1-oxide from (*N*-2-picolyl 1-oxide)formamide by usual methods[12a] such as the phosphoryl-pyridine method, the trialkyl amine method, and the cyanuric chloride-K_2CO_3 method, have always failed.

triphenylphosphine and carbon tetrachloride at 60°, according to the procedure of Appel and co-workers.[13]

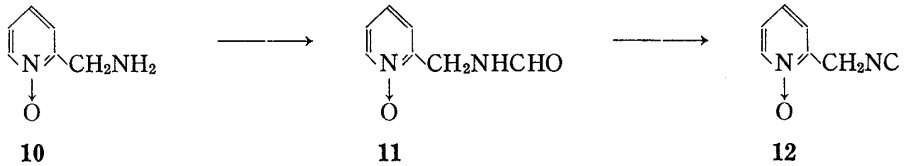
The reaction of **3** with bromoacetaldehyde diethyl acetal in the presence of potassium carbonate in DMF for one week afforded 1-(2-picolyl 1-oxide)-3-(2,2-diethoxyethyl)uracil (**4**) in 91% yield. Upon acidic hydrolysis, an equimolar mixture of 1-(2-picolyl 1-oxide)-3-formylmethyluracil (**5a**) and its methyl hemiacetal (**5b**) was obtained in 96.8% yield, after silica gel chromatography. The mixture of **5a** and **5b** was used for the subsequent condensation.

Initially, the four-component condensation involving **5a** and **5b** was carried out with commercially obtained cyclohexyl isocyanide as an isocyanide component. Thus, the above equimolar mixture was allowed to react with 2-picolylamine 1-oxide, cyclohexyl isocyanide, and acetic acid in methanol for 2 hr at room temperature. After work-up, a crystalline condensation product (**6**) was isolated by column chromatography on silicic acid in 82.7% yield. The structure was comfirmed by the combustion values and by spectral (UV and NMR) analysis.

For the deblocking of the two 2-picolyl 1-oxide groups, compound (**6**) was heated with excess acetic anhydride in DMF at 45° for 48 hr, and the resulting acetate (**7**) was treated with ammonium hydroxide at room temperature to effect the hydrolysis of acetates. After work-up, including column chromatographic purification, 3-(2-*N*-cyclohexylcarbamoyl-2-acetamidoethyl)uracil (**8**) was obtained in 63.9% yield. Its combustion values are consistent with the structure assigned. The ultraviolet absorption maximun in aqueous solution appeared at 261 nm, whereas in basic media the maximum shifted to 287.5 nm, accompanied by a hyperchromic change, indicating that the product was indeed 1-unsubstituted 3-alkyluracil.

Acidic hydrolysis of **8** with 6 N hydrochloric acid at 80° in a sealed tube afforded two ninhydrin-positive products (**1** and **9**). They were separable from one another by a combination of recrystallization and DEAE-cellulose column chromatography. Thus, after the usual work-up of the hydrolysate, crystallization from aqueous ethanol afforded 3-(2-*N*-cyclohexylcarbamoyl-2-aminoethyl)uracil (**9**). The structure was confirmed by the combustion values and by UV spectroscopy. (±)-Isowillardiine (**1**) was isolated by DEAE-cellulose column chromatography in 25.2% yield. The purity was checked by paper chromatography in three different solvent systems and by paper electrophoresis at pH 7.5. Its structure was confirmed by spectral (UV and NMR) analysis, as well as by the combustion values. It should be noted that the synthetic racemic sample was found to be identical with a natural sample of isowillardiine provided by Dr. Isamu Murakoshi on the criteria of *Rf* values on paper chromatography (in three different solvent systems), UV spectra and pK_a values.

The synthesis of (±)-isowillardiine (**1**) could also be achieved by a one-pot "four-component condensation," involving 2-picolyl isocyanide 1-oxide in place of cyclohexyl isocyanide.



10 **11** **12**

Chart 2

Experimental

General——The melting points are uncorrected and were taken on a Yamato melting point apparatus. The NMR spectra were recorded on a Hitachi NMR spectrometer, model R24, and the following abbreviations are used: s, singlet; d, doublet; bd, broad doublet; TEAB, tetraethylammonium bicarbonate. The chemical

13) R. Appel, R. Kleinstück, and K.-D. Ziehn, *Angew. Chem., Internat. Edit.*, **10**, 132 (1971).

shifts are given in parts per million downfield from Me_4Si. The mass spectrometer employed was a Hitachi RMU-6E instrument, at an ionizing energy of 80 eV. Elemental analyses were performed by the staff of the Analytical Center of Hokkaido University.

1-(2-Picolyl 1-Oxide)-3-(2,2-diethoxyethyl)uracil (4)——Bromoacetaldehyde diethyl acetal (5.52 g, 28 mmol) was added to a suspension of 1-(2-picolyl 1-oxide)uracil (3) (4.38 g, 20 mmol) and K_2CO_3 (3.87 g, 28 mmol, 1.4 equiv.) in DMF (100 ml). The mixture was stirred for seven days at 90° (bath temperature). The mixture was taken to dryness under reduced pressure, and the residue was partitioned between $CHCl_3$ and water. The organic layer was concentrated to dryness and applied to a silica gel column (silica gel, 140 g). The column was washed with $CHCl_3$–EtOH (1000: 50), and fractions containing **4** were pooled. Removal of the solvent left **4** as a foam. Yield, 6.10 g (91%). NMR (δ in $CDCl_3$): 1.10 (6H, t, $-OCH_2\underline{CH}_3\times2$), 3.55 (4H, m, $-O\underline{CH}_2CH_3\times2$), 4.05 (2H, d, $\underline{CH}_2N^3$), 4.85 [1H, t, $(EtO)_2\underline{CH}$], 5.05 (2H, s, CH_2-N^1).

1-(2-Picolyl 1-Oxide)-3-(formylmethyl)uracil (5a)——A solution of **4** (6.0 g, 17.9 mmol) in ethanol (10 ml) was treated with 0.5 N HCl (20 ml, 0.56 equiv.). The solution was heated at 70° for 1 hr, then neutralized with $NaHCO_3$ and concentrated. The residue obtained by removal of the ethanol was then applied to a silica gel column (silica, 120 g). The column was washed with $CHCl_3$–MeOH (1000: 50) and the fractions containing **5a** and **5b** were pooled. Removal of the solvent left the product as a foam. The NMR spectrum of the foam in $CDCl_3$ showed the presence of a signal (δ 3.25 ppm) due to methyl hemiacetal (integration value, 1.5) and a signal (δ 9.45 ppm) due to a formyl proton (integration value, 0.5). On the basis of the NMR spectral analysis, the product(s) were identified as a 1: 1 mixture of an aldehyde derivative (**5a**) and the corresponding methyl hemiacetal (**5b**). Crystallization of a portion of the mixture from ethanol afforded an analytical sample of **5a**, mp 186—189°. IR ν_{max}^{Nujol} cm^{-1}: 1720. *Anal.* Calcd for $C_{12}H_{11}N_3O_4$: C, 55.17; H, 4.24; N, 16.09. Found: C, 55.02; H, 4.22; N, 16.01.

Four-Component Condensation (Ugi Reaction) involving 5a and 5b, (2-Picolyl 1-Oxide)amine, Cyclohexyl Isocyanide and Acetic Acid. Synthesis of the Ugi Product (6a)——Cyclohexyl isocyanide (787 mg, 7.22 mmol) was added with stirring to a solution of a 1: 1 mixture of **5a** and **5b** (2.0 g, 7.22 mmol), 2-picolylamine 1-oxide[11] (895 mg, 7.22 mmol) and acetic acid (866 mg, 14.4 mmol) in methanol (25 ml). The stirring was continued at room temperature until **5a** and **5b** were consumed (the reaction was monitored by TLC, using $CHCl_3$–MeOH 7: 1). It required about 2 hr. The solvent was then removed *in vacuo*. The residue was applied to a silica gel column (silica, 100 g). The column was initially washed with $CHCl_3$–MeOH (1000: 50); this eluate was discarded and the column was then washed with $CHCl_3$–MeOH (1000: 100). The eluate was pooled and concentrated to dryness. Recrystallization from ethanol afforded an analytical sample, mp 240—242° (dec.). Yield, 3.2 g (82.7%) UV $\lambda_{max}^{H_2O}$ 258.5 nm. The ultraviolet maximum did not shift in acidic or alkaline media. *Anal.* Calcd for $C_{27}H_{32}N_6O_6$: C, 60.43; H, 6.01; N, 15.66. Found: C, 60.15; H, 6.01; N, 15.42.

Synthesis of 8. Deblocking of 6a by Acetic Anhydride Treatment and Hydrolysis——The above Ugi product (**6a**) (2.0 g, 3.7 mmol) was dissolved in DMF (10 ml) and acetic anhydride (10 ml). The solution was heated at 45° for 48 hr, then excess acetic anhydride was removed *in vacuo*. The residue was dissolved in 10% NH_4OH solution (10 ml). The solution was kept at room temperature for 15 min, then concentrated. The residue was triturated with ethanol and filtered, and the solid was collected (yield, 410 mg). The filtrate was concentrated to dryness and applied to a silica gel column, using $CHCl_3$–EtOH (1000: 80). From the eluate, 350 mg of the product was obtained after removal of the solvent. The combined yield was 760 mg (63.9%). Recrystallization from ethanol afforded an analytical sample, mp 250—252° (dec.). UV $\lambda_{max}^{H_2O}$ 261 nm. In basic media, the UV maximum shifted to 287.5 nm. *Anal.* Calcd for $C_{15}H_{22}N_4O_4$: C, 55.88; H, 6.88; N, 17.38. Found: C, 55.87; H, 6.92, N, 17.36.

Acidic Hydrolysis of 8. Formation of (±) Isowillardiine (1) and the Corresponding N-Cyclohexylcarboxamide (9)——A suspension of **8** (200 mg) in 6 N HCl (8 ml) was heated in a sealed vessel at 80° for 25 hr, during which period the solid gradually dissolved. Upon electrophoresis of the reaction mixture in 0.05 M TEAB solution (at 80 V/cm for 1.5 hr), one (**9**) of two ninhydrin-positive products had a mobility of 1.3 cm and the other had a mobility of 7.8 cm, compared to a mobility of 7.8 cm for isowillardiine. The reaction mixture was concentrated to half its original volume and methanol was added to deposit a crystalline compound, which was recrystallized from aq. ethanol, mp 245—247° (dec.). UV $\lambda_{max}^{H_2O,orpH2}$ 261 nm, $\lambda_{max}^{0.1\ NNaOH}$ 287nm. *Anal.* Calcd for $C_{13}H_{20}N_4O_3\cdot HCl\cdot 1/2H_2O$: C, 47.93; H, 6.76; N, 17.20. Found: C, 47.96; H, 6.68; N, 17.29.

The filtrate was adjusted to pH 8 and applied to a DEAE-cellulose column (wet volume of DEAE, 150 ml). The column was washed with H_2O. The eluate was concentrated, and crystallization of the residue from H_2O afforded an analytical sample. Yield of **1** was 31 mg (25.2%), mp 226° (dec.). UV $\lambda_{max}^{pH\ 2}$ nm (ε): 261 (7500); $\lambda_{max}^{pH\ 12}$ nm (ε) 286 (10400): *Rf* in PPC (BuOH–AcOH–H_2O 60 :15: 25); 0.20 (uracil 0.52): *Rf* in EtOH–H_2O (8: 2); 0.13 (uracil 0.48): in EtOH–c NH_4OH–H_2O (8: 1: 2); 0.41 (uracil 0.54). PEP in $Et_3\overset{+}{N}HCO_3^-$ (0.05 M); 6.2 cm **1** compared to 2.3 cm (uracil). *Anal.* Calcd for $C_7H_9N_3O_4\cdot 1/4H_2O$: C, 41.27; H, 4.70; N, 20.63. Found: C, 41.13; H, 4.70; N, 20.55.

N-(2-Picolyl 1-Oxide) Formamide (11)——A solution of 2-picolylamine 1-oxide[11] (12.4 g, 0.1 mol) in ethyl formate (100 ml) was refluxed for 9 hr. On thin-layer chromatography, a spot corresponding to "op"

amine disappeared and a single product was formed almost exclusively. The reaction mixture was concentrated to half its original volume. The crystals which deposited were collected and recrystallized from ethanol, mp 147—148°. *Anal.* Calcd for $C_7H_8N_2O_2$: C, 55.26; H, 5.36; N, 18.41. Found: C, 55.19; H, 5.36; N, 18.43.

2-Picolyl Isocyanide 1-Oxide (12)——N-(2-Picolyl 1-oxide)formamide (10.64 g, 70 mmol), carbon tetrachloride (10.77 g, 70 mmol), triethylamine (7.08 g, 70 mmol), and triphenylphosphine (20.03 g, 84 mmol) were dissolved in chloroform (100 ml). The solution was heated at 60° for 3 hr, then the solvent was distilled off under reduced pressure and the residue was partitioned between CCl_4 (100 ml) and H_2O (100 ml). The organic layer was separated and washed with H_2O (100 ml). The combined aqueous layers were concentrated and the residue was dried by co-distillation with ethanol. The final residue was dissolved in a small amount of chloroform and applied to a silica gel column (silica, 400 g). The column was washed with $CHCl_3$–EtOH (95: 5); fractions containing the isocyanide were pooled, and concentrated to dryness. Crude yield, 26.65 g (70.9%). Washing the residue with a small volume of chloroform afforded an analytical sample, mp 139—141°. IR ν_{max}^{Nujol} (cm^{-1}): 2150, NMR (δ in $CDCl_3$): 4.93 (2H, s), 7.40 (3H, m), 8.25 (1H, t), MS *m/e*: 134 (M^+), 106 (M^+-28). *Anal.* Calcd for $C_7H_6N_2O$: C, 62.68; H, 4.51; N, 20.89. Found: C, 62.52; H, 4.33; N, 20.91.

One Pot (and Improved) Synthesis of (±)-Isowillardiine (1). Four-Component Condensation involving 2-Picolyl Isocyanide 1-Oxide (12)——2-Picolylamine 1-oxide (250 mg, 2 mmol), AcOH (225 mg, 3.75 mmol), 2-picolyl isocyanide 1-oxide (269 mg, 2 mmol), and three pieces of Linde molecular sieve 4A were added to a solution of an equimolar mixture of **5a** and **5b** (524 mg, 2 mmol) in absolute methanol (10 ml), and the mixture was stirred at room temperature for two days. After ascertaining by TLC ($CHCl_3$–MeOH 5: 1) that the reaction was completed, the reaction mixture was concentrated, and the residue was applied to a silica gel column (silica, 25 g). The column was washed with $CHCl_3$–MeOH (10: 1). Fractions containing the product were pooled, and removal of the solvent left a crude sample of the condensation product. This was dissolved in a small quantity of chloroform and insoluble material was filtered off. Removal of the solvent left a gummy substance (890 mg).[14] A portion (300 mg) of this was dissolved in acetic anhydride (2 ml) and the solution was heated at 40° overnight. Concentration of the mixture afforded a residue, which was applied to a silica gel column (silica, 10 g). The column was washed with $CHCl_3$–MeOH (95: 5) and fractions containing **7** were pooled. The mixture was concentrated and the residue was dissolved in 16 ml of 28% NH_4OH–ethanol (1: 3 v/v). The solution was kept at room temperature for 4 hr. The solvent was then removed, and the residue obtained was dissolved in 5 N HCl (20 ml). This solution was heated for 3 hr in a boiling water bath, then the solvent was removed *in vacuo* and the residue was dissolved in pyridine. The pH of the solution was adjusted to *ca.* 6.5 and 1 ml of ethanol was added. During storage, crystals were deposited and were subsequently collected. The yield of **1** was 10.5 mg. The mother liquor was adjusted to pH 10 with 5% NH_4OH, and the resulting solution was applied to a DEAE-cellulose column (3 × 40 cm). The column was eluted initially with H_2O (180 ml) and then with a linear gradient system (0→0.2 M TEAB solution), collecting fractions of 18 ml. Fractions 50—100 were pooled, concentrated to a small volume and rechromatographed in the same way. Fractions containing **1** were concentrated to dryness. Recrystallization from aq. EtOH afforded crystals of **1**. Yield, 102 mg. The combined crystals (10.5 mg and 102 mg) were recrystallized from H_2O to afford an analytical sample. Yield, 92 mg. mp 226° (dec.). This sample was found to be identical with the authentic sample described above.

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14) Based on TLC examination and NMR analysis, this gummy substance was found to contain a considerable amount of an unusual Ugi product which after deblocking and hydrolysis gave rise to (±)-isowillardiine (**1**). The structural determination and the mode of formation of this product will be the subject of a separate paper.