# Structure and Synthesis of Histopine, a Histidine Derivative Produced by Crown Gall Tumors†

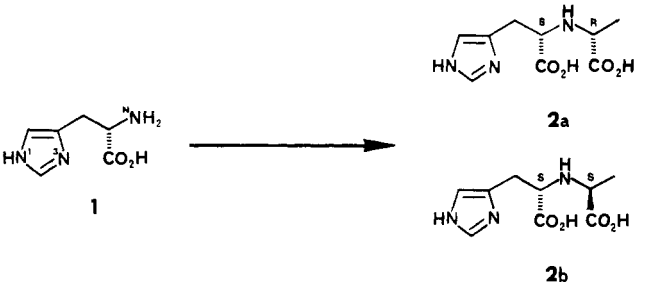
Hans Aaron Bates,* Alka Kaushal, Ping-Nan Deng, and Daniela Sciaky

ABSTRACT: Histopine, an unusual amino acid derivative of histidine isolated from crown gall tumors of sunflowers (*Helianthus annus*) inoculated with *Agrobacterium tumefaciens* strain $B_6$, was previously assigned the gross structure *N*-(1-carboxyethyl)histidine (**2**). A diastereomeric mixture containing histopine (**2a** and **2b**) was readily prepared by reductive alkylation of (*S*)-histidine (**1**) with pyruvic acid and sodium cyanoborohydride. The individual diastereomers were prepared by reaction of (*S*)-histidine with (*R*)- and (*S*)-2-bromopropionic acid. (*R*)-*N*-(1-Carboxyethyl)-(*S*)-histidine (**2a**) supports the growth of *A. tumefaciens* whereas (*S*)-*N*-(1-carboxyethyl)-(*S*)-histidine (**2b**) is inactive. Therefore, we assign structure **2a** to histopine.

Crown gall tumors are caused by infection of dicotyledonous host plants by the bacterium *Agrobacterium tumefaciens*. Examination of transformed plant cells has shown that a fraction of the *A. tumefaciens* Ti plasmid is integrated into the host genome where it directs the synthesis of products not normally found in the plant (Bevan & Chilton, 1982). This phenomenon may be viewed as an example of natural genetic manipulation (Chilton et al., 1977; Drummond et al., 1977). When infected by *A. tumefaciens*, the dicotyledonous plant cells synthesize abnormal *N*-(1-carboxyethyl) amino acid derivatives called opines that cannot be metabolized by the plant but are utilized by the parasite as nutrients (Drummond, 1979; Tempé & Goldmann, 1982). The nature of the abnormal amino acid metabolite formed is determined by the strain of *A. tumefaciens* that causes the tumor.

Histopine, an unusual derivative of the amino acid histidine (**1**), was isolated from crown gall tumors of sunflowers (*Helianthus annus*) inoculated with *A. tumefaciens* strain $B_6$ (Kemp, 1977, 1978). Histopine, which is found in octopine crown gall tumors (Kemp, 1977), is a unique opine in that it does not induce octopine catabolism in *A. tumefaciens* (Petit & Tempé, 1978). Although the quantity isolated was insufficient for a rigorous structure determination, histopine was assigned the gross structure **2** on the basis of incorporation of labeled (*S*)-L-histidine and pyruvic acid, analogy with related *N*-(1-carboxyethyl) amino acid derivatives (opines) isolated from crown gall tumors, and the mass spectrum of its ethyl ester. Interestingly, **2** had previously been encountered in an unrelated investigation in which the Schiff base intermediate formed between histidine and *Lactobacillus* histidine decarboxylase was reduced with sodium cyanoborohydride (Recsei & Snell, 1970). The diastereomers of histopine were recently separated and characterized by ORD (Kitajima et al., 1982).

In this paper, we report the synthesis of two diastereomers of *N*-(1-carboxyethyl)-(*S*)-histidine (**2a** and **2b**). (*R*)-*N*-(1-Carboxyethyl)-(*S*)-histidine (**2a**) supports the growth of *A. tumefaciens* whereas (*S*)-*N*-(1-carboxyethyl)-(*S*)-histidine (**2b**) is inactive. Therefore, we assign structure **2a** to histopine.




## Materials and Methods

*General.* Reactions were stirred magnetically. Solutions were evaporated in vacuo with a rotary evaporator at 55 °C or less. $^1H$ NMR spectra in $D_2O$ were recorded on Varian EM-360 (60 MHz) and HFT-80 (80 MHz) spectrometers. Chemical shifts are expressed in parts per million downfield from a DSS internal standard. $^{13}C$ NMR spectra were recorded on a Varian CFT-20 spectrometer (20 MHz) in $H_2O$ and $D_2O$ with dioxane ($\delta$ 67.39) as an internal standard. Samples for NMR were prepared by evaporation of $NH_4OH$ solutions, unless otherwise noted. Ion-exchange column chromatography resin, AG 50W-X8 (100–200 mesh), was preequilibrated with 2 M HCl and eluted with 0.6 M $NH_4OH$. Unless noted otherwise, the column was 1.1 cm in diameter by 10.3 cm in length. Thin-layer chromatography (TLC) was performed on silica gel covered aluminum plates. Plates were eluted with *t*-BuOH–$H_2O$–pyridine (50:47:3) and then visualized with $I_2$ or Pauly reagent (Stahl, 1969). The $I_2$ chamber detects histidine ($R_f$ = 0.12), histopine ($R_f$ = 0.31), and the imidazole-alkylated side product (or 2-bromopropionic acid, $R_f$ = 0.72), but Pauly reagent only visualizes two spots, histidine and histopine (Fournari et al., 1968).

*High-Pressure Liquid Chromatography.* High-pressure liquid chromatography (HPLC) was carried out on a Waters 30 × 0.39 cm micro-C18 column with a Perkin-Elmer LC-55 detector adjusted to 212 nm. The column was eluted at 2 mL/min with 0.138 M triethylammonium phosphate buffer prepared from water (1 L), $H_3PO_4$ (3.0 mL, 85%), and triethylamine (8 mL to pH 3.9) and brought to a boil before use. Typical elution times in minutes were as follows: histidine, 1.76; histopine, 1.97; lactic acid, 2.33; imidazole-alkylated side product, 2.90; 2-bromopropionic acid, 3.89.

*Reductive Alkylation of* L-*Histidine Hydrochloride with Pyruvic Acid and Sodium Cyanoborohydride.* A solution of

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L-histidine–HCl–$H_2O$ (125 mg, 0.596 mmol) and pyruvic acid (264 mg, 3.0 mmol, 500 mol %) in 10 mL of distilled water was adjusted with 5% sodium bicarbonate (approximately 8 mL) to pH 7.0, and sodium cyanoborohydride (118 mg, 1.88 mmol, 314 mol %) was added. The solution was stirred for 48 h at room temperature, 0.6 mL of concentrated HCl was added, and the solution was stirred 1 h. The solution was concentrated to 3.5 mL and then was absorbed onto an AG 50W-X8 ion-exchange column equilibrated with 2 N HCl. After being washed with 250 mL of $H_2O$, the product was eluted with 0.6 N $NH_4OH$ (approximately 40 mL). The eluent was rotary evaporated to dryness to give 130 mg (0.572 mmol, 95% yield) of **2a** and **2b** as a white solid: mp 235 °C dec; HPLC showed a single peak; $^1H$ NMR and $^{13}C$ NMR, see below; $[\alpha]^{25}_D$ +15.6° (*c* 24 mg/mL, 1 M HCl).

*(S)-2-Bromopropionic Acid (Fu et al., 1954).* (*S*)-L-Alanine (3.56 g, 39.95 mmol) was dissolved in 6 M HBr (50 mL, 300 mmol, 750 mol %), cooled to 0 °C, and stirred vigorously. Sodium nitrite (4.40 g, 63.8 mmol, 160 mol %) was added at such a rate that the temperature remained below 5 °C. After 18 h more at 5 °C, the solution was extracted 3 times with ether. The ether extract was dried over sodium sulfate for 0.5 h, and the solvent was evaporated to afford the crude product (2.34 g). This was purified by Kugelrohr distillation at 120 °C under 4 mmHg [lit. bp 78 °C (4 mm), conventional distillation] to afford the product (2.06 g, 34% yield) as a colorless liquid, which was pure by gas chromatography and $^1H$ NMR: $[\alpha]^{25}_D$ −39.95° [lit. −46° (Fu et al., 1954)]. No significant decrease in optical rotation occurred after 1 week at 20 °C.

*(R)-2-Bromopropionic acid* was prepared as above from (*R*)-D-alanine.

*Alkylation of 1-Benzyl-L-histidine with Racemic 2-Bromopropionic Acid and Barium Hydroxide at pH 10.* A solution of 1-benzyl-L-histidine (Sigma, 100 mg, 0.408 mmol) and racemic 2-bromopropionic acid (125 mg, 0.815 mmol, 200 mol %) in 5 mL of $H_2O$ was stirred at 55 °C while the solution was maintained at pH 10.0 with 0.233 M $Ba(OH)_2$ with an autotitrator. After 71.5 h, the still incomplete reaction was treated with $H_2SO_4$ and purified by ion-exchange chromatography as before. HPLC showed the presumed product (7.27 min), a side product (12.4 min), and 1-benzyl-L-histidine (4.16 min).

*Alkylation of (S)-L-Histidine with Racemic 2-Bromopropionic Acid and Barium Hydroxide (No pH Control).* A solution of L-histidine–HCl–$H_2O$ (1.25 g, 5.96 mmol), racemic 2-bromopropionic acid (940 mg, 6.14 mmol, 103 mol %), and anhydrous barium hydroxide (2.06 g, 12.0 mmol, 200 mol %) in $H_2O$ (50 mL) was stirred 22 h at 55 °C. Concentrated $H_2SO_4$ (0.7 mL, to pH 1.3) was added and the insoluble barium sulfate was removed by centrifugation. The clear filtrate was applied to a column of AG 50W-X8 (1 × 10 cm, 100–200 mesh) ion-exchange resin equilibrated with 2 M HCl. The column was eluted with water (350 mL) until the pH of the eluent was 4.1 and then with 0.6 M $NH_4OH$. Fractions that contained histidine and *N*-(1-carboxyethyl)histidine (**2**) (40–70 mL of 0.6 M $NH_4OH$) were combined, and the solvent was evaporated to afford a white solid (1.24 g). Integration of the $CH_3$ groups in the $^1H$ NMR showed a 48:41:11 mixture of histidine, *N*-(1-carboxyethyl)histidine (**2**), and imidazole-alkylated side product. A portion of this mixture (200 mg) was applied to a silica gel (100–200 mesh) column (3 × 18 cm) which was eluted with *t*-BuOH–water–pyridine (50:47:3). The fractions that contained the product (105–150 mL) were evaporated and applied to a column of AG 50W-X8 (1 × 10 cm) which was eluted as before to afford **2a** and **2b** (70 mg, 32% yield) as a white solid: 97% pure by HPLC; mp 235 °C dec. This material was identical (NMR and HPLC) with that prepared by reductive alkylation with pyruvic acid.

*(R)-N-(1-Carboxyethyl)-(S)-histidine* (**2a**, *Histopine*). (*S*)-L-Histidine was alkylated with (*S*)-2-bromopropionic acid and barium hydroxide (no pH control) exactly as described above, and the product was purified to afford **2a** (32% yield) as a white solid: 97% pure by HPLC; $[\alpha]^{25}_D$ +17.6° (*c* 24 mg/mL, 1 M HCl); $^1H$ NMR $\delta$ 1.37 (3 H, d, $CH_3$), 3.17 (2 H, d, $CH_2$), 3.65 (2 H, m, CH), 7.1 (1 H, s, im), 7.9 (1 H, s, im); $^{13}C$ NMR $\delta$ 15.63 ($CH_3$), 27.88 ($CH_2$), 58.21 (*C*HC-$H_3$), 61.50 (*C*H$CH_2$), 118.14 (C-5), 131.96 (C-2), 136.59 (C-4), 175.75 (COOH); mp 248 °C dec.

*(S)-N-(1-Carboxyethyl)-(S)-histidine* (**2b**). (*S*)-L-Histidine was alkylated with (*R*)-2-bromopropionic acid and barium hydroxide, and the product was purified as described above to afford **2b** (32% yield) as a white solid: mp 214 °C; $[\alpha]^{25}_D$ +14.7° (*c* 24 mg/mL, 1 M HCl); $^1H$ NMR $\delta$ 1.45 (3 H, d, $CH_3$), 3.17 (2 H, d, $CH_2$), 3.6 (2 H, m, CH), 7.15 (1 H, s, im), 8.1 (1 H, s, im); $^{13}C$ NMR $\delta$ 16.88 ($CH_3$), 28.19 ($CH_2$), 58.91 (*C*H$CH_3$), 62.47 (*C*H$CH_2$), 117.90 (C-5), 131.46 (C-2), 136.59 (C-4), 173.73 (COOH); mp 215 °C dec.

*α-Methyl-1H-imidazole-1-acetic Acid Hydrochloride* (**3**). *Method A.* A solution of imidazole (0.500 g, 7.35 mmol), 2-bromopropionic acid (1.15 g, 7.52 mmol, 102 mol %), and barium hydroxide (2.50 g, 14.6 mmol, 199 mol %) in water (20 mL) was stirred at 55 °C for 26 h. The mixture was cooled and concentrated $H_2SO_4$ (0.88 mL) was added (pH 1.7). The barium sulfate was removed by centrifugation, and the water was evaporated from the supernatant to afford crude product (1.56 g). A portion (100 mg) was chromatographed on AG 50W-X8 in the manner described above and then chromatographed on AG 1-X2 (5 × 0.6 cm column, chloride form, −400 mesh, previously equilibrated with 0.6 M $NH_4OH$). The column was eluted with water (25 mL) until the pH reached 7, and the product was eluted with 0.1 M HCl (20 mL). Evaporation of the solvent afforded the HCl salt of **3** (51 mg, 61% yield) as an off-white glass.

*Method B (Fournari et al., 1968).* The potassium salt of imidazole (20.0 mmol) and ethyl 2-bromopropionate (2.50 mL, 3.62 g, 20 mmol, 100 mol %) were refluxed in benzene (30 mL) for 10 h to afford ethyl α-methyl-1*H*-imidazole-2-acetate (448 mg, 13% yield) as a pale yellow liquid. This ester was hydrolyzed with 1 M NaOH (150 mol %) for 23 h at 20 °C. The excess base was neutralized with HCl (to pH 2), and the water was evaporated. Absolute ethanol was added to the residue, and the supernatant was evaporated to afford the HCl salt of **3** (71% yield) as an off-white glass: $^1H$ NMR $\delta$ 1.82 (3 H, d), 5.03 (1 H, q), 7.45 (2 H, d), 8.65 (1 H, br s); $^1H$ NMR ($NH_4^+$ salt) $\delta$ 1.71 (3 H, d), 4.90 (1 H, q), 7.35 (2 H, m), 8.45 (1 H, m).

*Bioassay.* Sterile conditions were employed throughout. The following strains of *A. tumefaciens* were utilized in the bioassays (Sciaky et al., 1978): A136, a cured derivative of strain C58 and therefore lacking a Ti plasmid; A208, containing pTiT37, a nopaline Ti plasmid in A136; A277, containing pTiB6806, an octopine Ti plasmid in Al36; A756, which is strain A277 but constitutive for octopine and histopine catabolism (a gift of E. W. Nester). Bacteria were grown overnight in YEP broth: yeast extract (10 g/L), peptone (10 g/L), and NaCl (5 g/L). The bacteria were washed with T buffer consisting of $K_2HPO_4$ (10.5 g/L) and $KH_2PO_4$ (4.5 g/L) to remove traces of carbon- and nitrogen-containing nutrients. Agar plates were prepared with filter-sterilized opine (1 mg/mL medium) mixed with minimal agar media (Petit

et al., 1978), which lacked a source of carbon and nitrogen. The bacterial strains were streaked across the agar surface, and the plates were incubated at 28–30 °C for 4 days. Bacteria were also streaked on agar without any source of carbon and nitrogen as well as on agar containing ammonium sulfate and mannitol.

## Results and Discussion

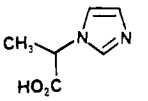
A diastereomeric mixture of (*R*)- and (*S*)-*N*-(1-carboxyethyl)-(*S*)-histidine, **2a** and **2b**, respectively, was readily prepared in 99% yield by reductive alkylation of (*S*)-L-histidine (**1**) and pyruvic acid (2-oxopropionic acid) with $NaBH_3CN$ (Borch et al., 1971; Jensen et al., 1977; Kitajima et al., 1982). The $^1H$ NMR and $^{13}C$ NMR spectra of the diastereomeric mixture indicated a 53 to 47 mixture of two diastereomers, subsequently determined to be **2a** and **2b**, respectively.

To evaluate the products and progress of reactions utilized to synthesize histopine, development of analytical chromatographic methods was necessary. Although separation of histidine (**1**) from *N*-(1-carboxyethyl)histidine (**2**) by thin-layer chromatography on cellulose had previously been reported (Kemp, 1977), we found that elution with the suggested solvent, *i*-PrOH–formic acid–water (40:2:10), did not separate **1** from **2** on a 20-cm cellulose plate, while elution with MeOH–water–pyridine (20:5:1) gave marginal separation with severe tailing. *t*-BuOH–water–pyridine (60:37:3) elution on a silica gel plate gave better separation, and we finally achieved good separation on silica gel with *t*-BuOH–water–pyridine (50:47:3). Analytical high-pressure liquid chromatography on a reverse-phase column gave good separation and was utilized routinely to follow reactions. TLC and HPLC separated histidine from *N*-(1-carboxyethyl)histidine (**2**) but did not separate to the two diastereomers **2a** and **2b**.

To prepare each diastereomer separately, we treated (*S*)-L-histidine with (*R*)- and (*S*)-2-bromopropionic acid. Preliminary experiments were performed with racemic 2-bromopropionic acid. Initially, we attempted to repeat the reported synthesis of *N*-(1-carboxyethyl)-(*S*)-histidine (**2**) by alkylation of (*S*)-L-histidine with 17 equiv of 2-bromopropionic acid in the presence of 3.6 equiv of aqueous barium hydroxide (Kemp, 1977). As expected, hardly any reaction occurred under the acidic conditions employed. Histidine was recovered in 99% yield, and HPLC analysis indicated that the yield of **2a** and **2b** was less than 0.5%. A 1:1:2 ratio of histidine hydrochloride, 2-bromopropionic acid, and barium hydroxide yielded 40% *N*-(1-carboxyethyl)histidine (**2**) but left 50% of the histidine unreacted. Rapid hydrolysis (complete in 3 h at 55 °C) of 2-bromopropionic acid to 2-hydroxypropionic acid (lactic acid) competed with the desired alkylation. It is noteworthy that hydrolysis seems to be more of a problem in the alkylation of histidine than in alkylation of other amino acids (Biellmann et al., 1977; Biemann et al., 1960; Izumiya et al., 1957; Herbst & Swart, 1946). Use of increased amounts of 2-bromopropionic acid and barium hydroxide did not change the yield appreciably, while use of less barium hydroxide (initial pH 3–9) gave no alkylation. Use of an autotitrator to maintain the pH at 9 or 10 with sodium hydroxide or barium hydroxide also failed to increase the yield of *N*-(1-carboxyethyl)histidine (**2**).

In addition to the problem of incomplete alkylation of histidine by 2-bromopropionic acid, HPLC and TLC analysis revealed the presence of a small amount of a side product, the $^1H$ NMR of which indicated alkylation on a nitrogen of the imidazole ring. Although, as a consequence of its basicity, the primary amine of histidine would be expected to be considerably more nucleophilic than the imidazole nitrogen atoms ($pK_a$ = 9.17 vs. 6.00), there is precedent for alkylation of imidazole under similar conditions (Sloan & Koch, 1983; Fletcher et al., 1979; Crestfield et al., 1966; Greenstein & Winitz, 1961).

To examine the facility with which imidazole alkylation occurs, imidazole itself was treated with 1 equiv of 2-bromopropionic acid in the presence of 2 equiv of aqueous barium hydroxide. After 26 h at 55 °C, a 61% yield of α-methyl-1*H*-imidazole-1-acetic acid (**3**) was produced, which was identical with a sample synthesized by an alternative method (Fournari et al., 1968). Similarity between the $^1H$ NMR of the side product from alkylation of histidine (doublet, δ 1.65) and that of **3** (doublet, δ 1.71) confirms that the side product arises from alkylation of the imidazole, presumably at N-1, away from the amino acid side chain.




**3**

We hoped that protection of the imidazole ring with a benzyl group would suppress this side reaction. However, alkylation of 1-benzylhistidine (Greenstein & Winitz, 1961) with 2-bromopropionic acid at pH 10 still gave two products, which apparently arise from alkylation of the primary amine and an imidazole nitrogen.

In view of these complications, we found that the most practical method for the preparation of the two diastereomers was reaction of (*S*)-histidine hydrochloride in water with 1 equiv of 2-bromopropionic acid and 2 equiv of barium hydroxide with subsequent separation of the product from the unreacted histidine and imidazole-alkylated side product by column chromatography. Initially when this approach was used with racemic 2-bromopropionic acid, a mixture of **2a** and **2b**, spectroscopically identical with that formed by reductive alkylation with $NaBH_3CN$ and pyruvic acid, was formed in 32% yield. Analogously, (*S*)-2-bromopropionic acid, which was prepared from (*S*)-alanine (Izumiya et al., 1957; Fu et al., 1954), afforded (*R*)-*N*-(1-carboxyethyl)-(*S*)-histidine (**2a**) and (*R*)-2-bromopropionic acid afforded (*S*)-*N*-(1-carboxyethyl)-(*S*)-histidine (**2b**). $^1H$ NMR and $^{13}C$ NMR readily distinguished the two diastereomers and established that each sample was isomerically pure.

Bioassays were performed in order to determine which of the diastereomers was capable of serving as the sole source of carbon and nitrogen for the bacterium *A. tumefaciens*. Assays were performed on a minimal agar medium that lacked both carbon and nitrogen sources (Petit et al., 1978). Strain A756, which can utilize histopine constitutively without induction by octopine, grew normally on media containing (*R*)-*N*-(1-carboxyethyl)-(*S*)-histidine (**2a**) or a mixture of (*R*)- and (*S*)-*N*-(1-carboxyethyl)-(*S*)-histidine (**2a** and **2b**), as well as octopine, but it failed to grow on medium containing (*S*)-*N*-(1-carboxyethyl)-(*S*)-histidine (**2b**) or on a control medium that lacked any source of carbon or nitrogen. The appropriate controls with strains A136, A208, and A277, which are unable to utilize histopine, failed to grow on any of the histopine agars. Although strain A277 does have a Ti plasmid with the ability to utilize histopine, it can only do so when induced with octopine.

These results establish that histopine is (*R*)-*N*-(1-carboxyethyl)-(*S*)-histidine (**2a**). The configuration of histopine is thus analogous to that of two other opines, octopine and octopinic acid, in which the basic amino acid fragment possesses the natural *S* configuration while the alanine portion possesses

the uncommon *R* configuration (Biellmann et al., 1977; Biemann et al., 1960).

Acknowledgments

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**Registry No.** **1**, 71-00-1; **2a**, 62504-27-2; **2b**, 85027-27-6; **3**·HCl, 90269-13-9; L-histidine hydrochloride, 645-35-2; (*S*)-2-bromopropionic acid, 32644-15-8; L-alanine, 56-41-7; (±)-2-bromopropionic acid, 10327-08-9; 1-benzyl-L-histidine, 16832-24-9; imidazole, 288-32-4; 2-bromopropionic acid, 598-72-1; pyruvic acid, 127-17-3; (*R*)-2-bromopropionic acid, 10009-70-8.

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# Structure and Stereochemistry of Succinamopine†

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ABSTRACT: *Agrobacterium tumefaciens* strains A518, A519, AT181, and EU6 incite crown gall tumors on tobacco that produce a new opine, succinamopine, not found in healthy tobacco. NMR, mass spectral analysis, synthesis, selective fermentation of diastereomers, and circular dichroism show that succinamopine is *N*-[(1*S*)-1-carboxy-2-carbamoylethyl]-(*R*)-glutamic acid (**1a**), formally the reductive conjugate of (*S*)-asparagine and α-ketoglutaric acid. Succinamopine is rapidly converted in acid into succinamopine lactam (**2a**) and finally into succinopine lactam (**2b**). Succinamopine and the two lactams are catabolized selectively by *A. tumefaciens* strains A518, A519, and A532 but not by the isogenic strain A136, which lacks the virulence plasmid.

The organism *Agrobacterium tumefaciens*, the causitive agent of crown gall in dicotyledonous plants, harbors a tumor-inducing (Ti) plasmid. A portion of the Ti plasmid (T DNA) is incorporated into the host plant genome and causes plant cells containing T DNA to proliferate as a cancerous growth independent of normal hormonal regulation. The T DNA contains a gene, expressed in the plant, that is responsible for production of one or more unusual metabolites called opines (Guyon et al., 1980). Most opines so far identified appear to be reductive condensation products of an L-amino acid (lysine, arginine, leucine, glutamine) and a carbonyl compound (pyruvic acid, α-ketoglutaric acid, mannose) (Biemann et al., 1960; Menagé & Morel, 1964; Goldmann et al., 1969; Firmin & Fenwick, 1978; Tate et al., 1982; Chang et al., 1983). The Ti plasmid also contains a gene, not a part of T DNA, expressed in the bacterium that permits the pathogenic bacterium to catabolize the particular opine that its plasmid causes the plant tumor to synthesize (Petit et al., 1970). Ti plasmids have been characterized by biochemical

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