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LATERAL ROOT INDUCING COMPOUNDS FROM THE BACTERIUM *ERWINIA QUERCINA*: ISOLATION, STRUCTURE AND SYNTHESIS

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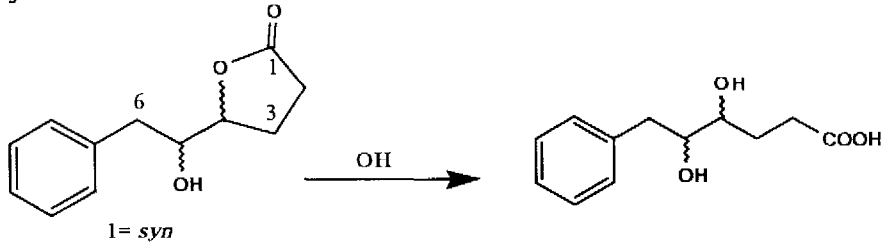
Summary: The structures lateral root inducing compounds produced in culture by the bacterium *Erwinia quercina* were shown to be two diastereomers of 4,5-dihydroxy-6-phenylhexanoic acid. All four chiral isomers of the active structure were synthesized.

During the investigation of a new bacterium, *Erwinia quercina*, found to be the causal agent of drippy nut disease of the California live oak, it was observed that inoculation of slices of carrots, turnips and beets with the bacterium induced the rapid development of copious quantities of lateral roots from root primordia.[2] It was also shown that a culture filtrate of the bacterium sterilized by passing it through a 0.22 μ membrane filter would cause identical root formation on carrot slices. We became interested in this observation and decided to isolate and characterize the compound(s) responsible for the observed biological activity. The isolation was guided by bioassay using the production of roots on carrot pieces[3] to follow the active compounds through the separation.

Concentrated culture filtrate[4] adjusted to pH 9 was passed thru a column of Dowex 1X8-200 anion exchange resin in the formate form. After elution with water the active material was eluted with 8 N formic acid. Further chromatography (MPLC, C-18, 20% CH_3CN-H_2O-0.075% CF_3COOH; HPLC, C-18, 10% CH_3CN-H_2O-0.075% CF_3COOH) gave peaks corresponding to two isomeric lactones (1 and 2). The lactones were an artifact of the isolation and were inactive in the bioassay; conversion to the acids was necessary for activity.



1= *syn*
2= *anti*

Analysis of ^{1}H and ^{13}C NMR, infrared, ultraviolet and mass spectra allowed for the assignment of the two diastereomers, structures 1 and 2. The main points of the analysis for isomer 2 follow. Exact mass determination of the molecular ion (observed m/z 206.0947, calculated m/z 206.0956) gave a molecular formula of $C_{12}H_{14}O_3$. Inspection of the ^{1}H and ^{13}C NMR spectra (CD_3OD) indicated the presence of a monosubstituted benzene ring [^{1}H NMR, δ7.26 (bs, 5H); ^{13}C NMR, δ139.40 (s), 130.46 (d, 2C), 129.44 (d, 2C), 127.41 (d)], two methine protons attached to carbons bearing oxygen [δ4.44 (dt, J=4,7, 1H) and 4.01 (m, 1H); ^{13}C 84.00 (d) and 73.99 (d)] and three methylene groups [^{1} δ2.77 (m, 2H), 2.59 (m, 2H), 2.26 (m, 2H); ^{13}C δ40.33 (t), 29.46 (t), 22.61 (t)]. The remaining carbon resonance at δ180.28 was determined to be a saturated γ-lactone by infrared absorption at 1778 cm^{-1}. Homonuclear decoupling experiments defined the chain of methylenes and methines existing in isomer 2 except for the relative positions of the carbonyl and benzene functional groups. This assignment was made on the basis of major ions in the mass spectrum at m/z 121 (M^{+}-$C_4H_5O_2$) and m/z 86 ($C_4H_6O_2$) of both isomers. These fragments could arise from cleavage of the bond between the two oxygen bearing carbon atoms in 2 but not from the isomeric structure in which phenyl and carbonyl groups are switched.

At this point a search of the literature revealed that the racemic diastereomers 1 and 2 had been synthesized during an investigation of related lactones which were components of the aroma of sherry wine.[5] Comparison of ^{1}H NMR, infrared and mass spectral data published in the synthetic study confirmed our gross structure assignments and allowed us to assign isomer 1, $[\alpha]_D$=24.1° $CHCl_3$, as <u>syn</u> and isomer 2, $[\alpha]_D$=-8.7° $CHCl_3$, as <u>anti</u>.

The questions of absolute stereochemistry remained to be solved. We decided to carry out a synthesis which would produce all four possible chiral isomers. The method chosen was based on the elegant work of Larcheveque and Lalande[6] on the synthesis of enantiomerically pure 1,2 diols. The synthesis was accomplished smoothly producing compounds A-D (Figure). The diastereomers were separated by HPLC (silica gel, hexanes/ethyl acetate/isopropanol, 55/35/10). In each case, the *anti* isomer was produced in excess over the *syn* isomer in a ratio of 70/30.

In preliminary testing all isomers had activity at concentrations of 10^{-3} to 10^{-4} M. Isomer D had the greatest activity, isomer C had the least activity. The natural materials 1 and 2 appear to be mixtures of enantiomers with B and C predominating respectively. The synthetic material gave an identical response to the natural material. The known auxins indole-3-acetic acid and β-naphthylacetic acid were active at concentrations of 10^{-2} and 10^{-3} M respectively in this assay.

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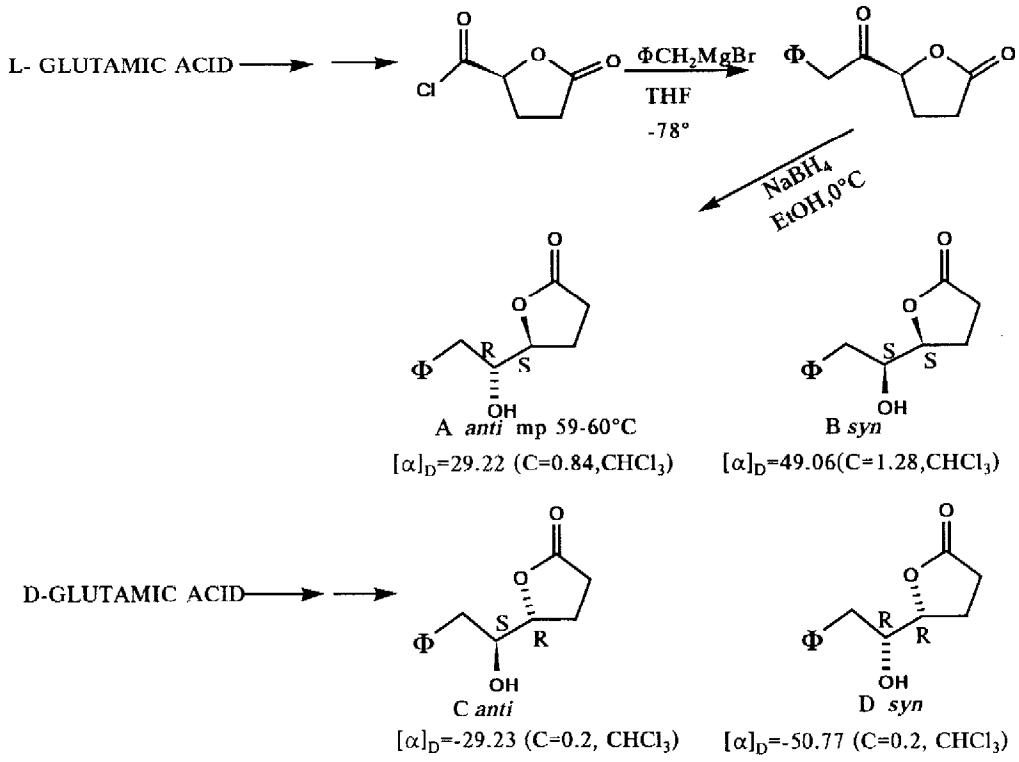

FIGURE

Table ^{13}C NMR Chemical Shifts of Compounds A-D in $CDCl_3$

Carbon No.	Compound A	B	C	D
3	21.49	23.90	21.42	24.00
2	28.46	28.53	28.44	28.59
6	38.90	39.86	38.87	39.93
5	72.47	74.30	72.42	74.37
4	81.87	81.15	81.89	81.15
10	126.70	126.73	126.63	126.84
9,11	128.60	128.64	128.54	128.74
8,12	129.20	129.33	129.17	129.38
7	136.97	137.12	136.99	137.06
1	177.57	177.55	177.62	177.50

References

1. Taken in part from the PhD dissertation by Amy Wright, University of California Riverside 1984.

2. D.C. Hildebrand and M.N. Schroth, Phytopathology, 57, 250, (1967). There is no known connection between the disease symptoms and the compounds produced in culture.

3. Fresh carrots purchased from a supermarket were washed with detergent and water, soaked for 15 minutes in a 1.9 (v/v) household bleach/distilled water solution and rinsed with sterile water. The carrots were cut into 1 inch long hemicylinders and placed flat surface down in sterile petri dishes containing a wet filter paper (3 pieces per dish, randomized). The sample to be assayed was dissolved in sterile water and pipetted onto the upper curved surface of the carrots. Three petri dishes were used per sample. The carrots were kept at room temperature (25°C) for 5-7 days at which time the results were recorded. Both positive and negative controls were run with each experiment. Development of lateral roots in 4 or more of the 9 pieces was taken as a positive response.

4. Cultures of *Erwinia quercina* were obtained from Professor M. Schroth, Dept. of Plant Pathology, University of California, Berkeley, CA. For isolation work the bacterium was grown in 500 ml of potato dextrose broth at 27°C on a shaker for 96 hours. The cultures were centrifuged, evaporated to approximately 50 ml. The concentrate was treated with ethanol to kill live cells and precipitate inactive material. After filtration and evaporation, the filtrate was ready for ion exchange. The two diastereomers were isolated in approximately a 1:1 ratio in a yield of 0.2-1 mg/L of culture.

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