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[2,3,4,6,6-$^2H_5$]-D-Glucose as a General Probe for Sugar Transformations in Microbial Metabolism: Application to the Biosynthesis of Sarubicin A, Blasticidin S, and Streptothricin F

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[2,3,4,6,6-$^2H_5$]-D-Glucose, **2c**, was shown to be a general probe for metabolism in *Streptomyces* leading to modified sugar units in secondary metabolites. Three antibiotics were studied in this regard: sarubicin A, blasticidin S, and streptothricin F—produced by *Streptomyces helicus, S. griseochromogenes,* and *S.* L-1689-23, respectively. Analysis of labeled antibiotics was by ^2H NMR spectroscopy. Incorporation of **2c** into sarubicin A resulted in deuterium enrichments at H-7_{cis} and at the methyl group, while incorporation of **2c** into blasticidin S resulted in deuterium enrichments at H-6, -7, -12, -14, and the *N*-methyl group. In each case the labelings could be interpreted by rational metabolisms. Incorporation of **2c** into streptothricin F led to extensive labeling of other portions of the molecule not directly derived from glucose, and the ^2H NMR spectrum was not interpretable.

INTRODUCTION

The metabolism of glucose leads to a wide variety of *O*-, *C*-, and *N*-glycoconjugates that are produced by microorganisms, higher plants, and animals (*2, 3*). These conjugates are formed as constituents of glycoproteins (*4*), glycolipids (*5*), and cell-wall components (*6*). In addition, there is a wide variety of antibiotics and other natural products that contain such conjugates. Often the sugar moiety is essential for the recognized biological activity. In these conjugates, glucose has been significantly transformed into members of such diverse structural groups as amino sugars, deoxy sugars, dehydro sugars, and aminocyclitols (*7*).

The pathways responsible for such glucose tranformations include oxidation, transamination, epimerization, dehydration, hydrogenation, and dehydrogenation reactions. At least some (*8*)—and perhaps many—of the enzymes that catalyze these reactions utilize sugar nucleosides as substrates. A typical example would be UDP-D-glucose isomerase (*9*). Formation of the 3,6-dideoxyhexose ascarylose

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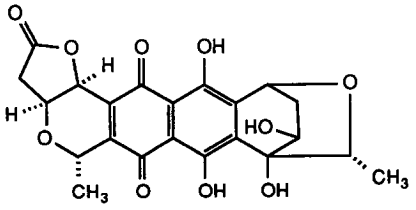
in *Pasteurella pseudotuberculosis* and related microorganisms is initiated from CDP-D-glucose (*10–13*), while phosphothymidine derivatives seem to be frequently used in *Streptomyces* (*14–16*).

Knowledge of which of the original carbinol hydrogens of glucose have been retained in any specific metabolite allows an appraisal of which reaction mechanisms would be acceptable for specific steps in its biogenesis. This has been established for biosynthesis of the sugar portions of granaticin (*15*), **1**, and chlorothricin (*16*). The approach used required a series of *in vivo* feedings with glucoses, each single-labeled with tritium, in each experiment admixed with ^{14}C-labeled glucose to provide an internal reference.



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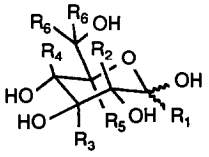
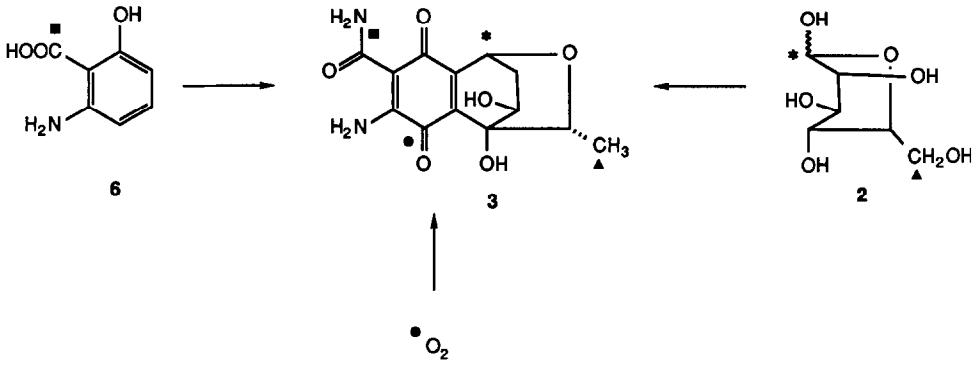
We have investigated the efficacy of using multiple-deuterated glucose with ^2H NMR analysis of the derived final metabolite to reveal in a single experiment which hydrogens have been retained. Perdeutero-D-glucose, **2a** (*17*), [2,3,4,5,6,6-$^2H_6$]-D-glucose, **2b** (*18*), and [2,3,4,6,6-$^2H_5$]-D-glucose, **2c** (*19*), have been prepared, but only the latter has been routinely commercially available (*20*). The use of these compounds has been quite limited (*21–23*); in no case was any used in a biosynthetic study. Three targets were chosen for our studies: sarubicin A, **3** (*24, 25*), blasticidin S, **4** (*26*), and streptothricin F, **5** (*27–29*); all three are antibiotics produced by *Streptomyces* species. The results reported here demonstrate that **2c** can be used effectively and is likely of general utility.



2a: $R_1 = R_2 = R_3 = R_4 = R_5 = R_6 = {}^2H$
2b: $R_1 = {}^1H, R_2 = R_3 = R_4 = R_5 = R_6 = {}^2H$
2c: $R_1 = R_5 = {}^1H, R_2 = R_3 = R_4 = R_6 = {}^2H$

RESULTS AND DISCUSSION

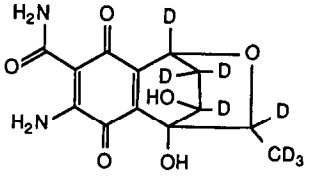
Sarubicin A, **3**, produced by *S. helicus*, was independently isolated and characterized by two groups, one at the Upjohn Co. Unpublished results (*30*) from the latter group had established the labeling pattern from [1-^{13}C]- and [6-^{13}C]-D-glucose, **2d** and **2e**, respectively, indicating that the bridged tetrahydropyran was derived intact from glucose, while we (*31*) had demonstrated that the quinone portion is derived from [^{13}COOH]-6-hydroxyanthranilic acid, **6**, and molecular oxygen, as shown in Scheme 1.



SCHEME 1

In order to study the origin(s) of the tetrahydropyranyl hydrogens of **3,** it was necessary to assign the ^{1}H NMR spectrum, in particular identifying which resonances belong to each of the methylene hydrogens. The proton spectrum of **3** in DMSO[2]-d_6 had been previously assigned (*32*), but we found that one of the hydrogen resonances had overlapped with the solvent resonance. However, in d_5-pyridine the methylene resonances had shifted only slightly from their positions in DMSO and both were now clearly visible (Fig. 1a).

Two 200-ml production broths (*31*) were prepared in 10% D_2O, and each was inoculated with 10 ml of a seed culture (*31*). After 72 h in an incubator–shaker (32°C, 225 rpm), workup (*31*) yielded 19.0 mg of pure **3a.** Analysis of the 61.4-



3a

MHz ^{2}H NMR spectrum (Fig. 1c) indicated deuterium enrichment at all positions and identified the line shape for each resonance. In the next fermentation (2 × 200 ml, no D_2O), a mixture of **2c** (135 mg) and [U-^{14}C]-D-glucose, **2f** (22.6 μCi), was divided between the two flasks 26.5 h after inoculation with the seed culture. The fermentations were terminated after a total of 72 h and worked up to yield 56.0 mg of pure **3b** (3.7% incorporation of ^{14}C). The ^{2}H NMR spectrum (Fig. 1b) showed resonances at δ 1.18 and 1.71 (integration, 3 : 1), indicating deuterium enrichment exclusively at the methyl and at H-7$_{cis}$ (stereochemistry relative to the hydroxyl at C-6). The results of this single biosynthetic experiment (**3b**) are fully consistent with the work of Floss *et al.* on granaticin (*15*): they indicate migration of deute-

[2] Abbreviation used: DMSO, dimethyl sulfoxide.

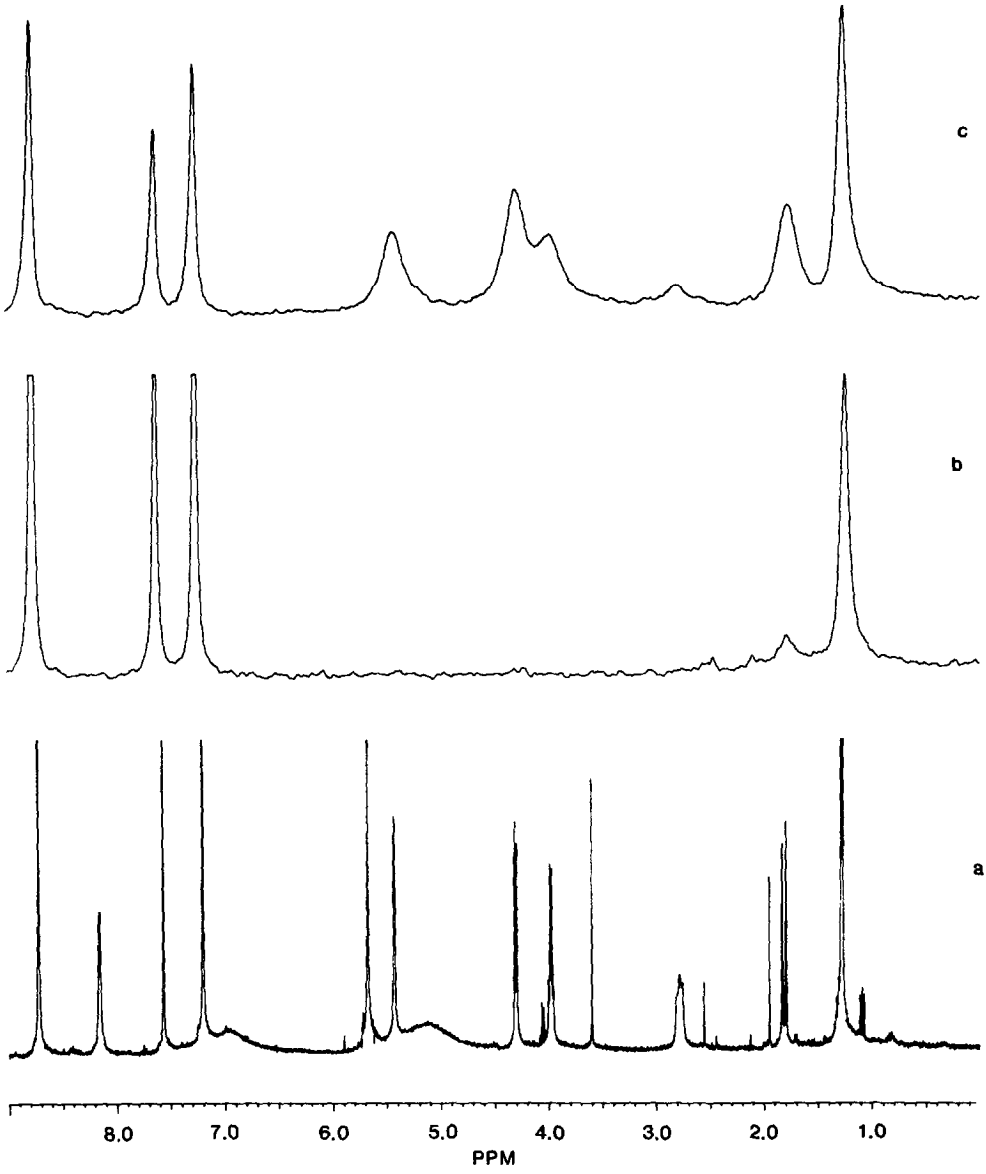
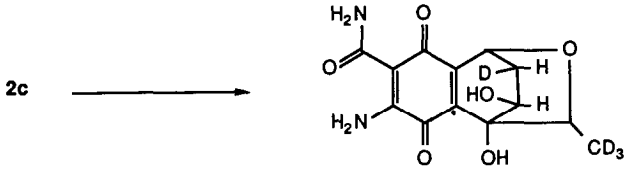


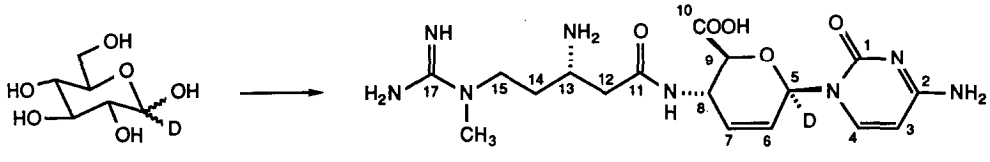
FIG. 1. (a) The 400-MHz ^1H NMR of sarubicin A **3**: SW 6024 Hz, SI = TD 16K, AQ 1.36s, NS 32, PW 3.0; (b) the 61.4-MHz ^2H NMR of sarubicin A **3b** from [2,3,4,6,6-$^2H_5$]glucose feeding: SW 952 Hz, SI 8K, TD 4K, AQ 2.15s, NS 16109, PW 13.5, LB 2 Hz; (c) the 61.4-MHz ^2H NMR of sarubicin A **3a** from D_2O feeding: NS 16380; all other parameters are the same as those for (b).

rium from C-4 to C-6 of glucose, retention of configuration at C-2 of glucose in the replacement of OH by H, loss of deuterium from H-3 of glucose consistent with a syn-elimination of water from C-2/C-3, and a syn-reduction of the double bond thus generated.



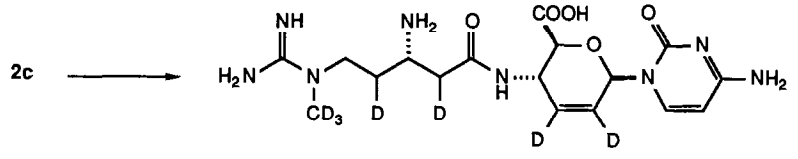
3b

Blasticidin S, **4,** produced by *S. griseochromogenes* is used commercially in Japan against *Piricularia oryzae* (rice blast). Prior to our work, Seto et al. (*33*) had established that α-arginine, D-glucose, cytosine, and methionine are the primary precursors to the skeleton of **4.** We have subsequently studied the conversion of α-arginine to the β-arginine moiety (*34, 35*). During the course of this latter work we completed the ^{1}H NMR spectral assignments (*34*). Prior to feeding **2c,** we established the fate of H-1 of glucose. Thus, [1-^{2}H]-D-glucose (*36*), **2g** (90.5 mg), was mixed with 18.0 μCi of **2f** and divided between two 250-ml production broths 44 h after they were inoculated with a seed culture (*34*). Incubation (29°C, 225 rpm) was continued for an additional 76 h and the fermentations were then worked up (*34*) to yield 11.0 mg of pure **4a** (bioassay of the crude broth after removal of solids indicated production of 74 mg of blasticidin for a 0.72% incorporation of **2f**). The 61.4-MHz ^{2}H NMR spectrum (Fig. 2b, deuterium-depleted water, 60°C to sharpen the resonances) contained a single peak from the sample at 6.55 ppm, corresponding to deuterium at H-5 (1.7% enrichment, 85% of that predicted, had **2g** been incorporated exclusively in the hexenuronic acid moiety), in addition to peaks from residual HOD (4.38 ppm) and from *t*-butanol (1.27 ppm) added as a chemical shift reference and for quantitation of deuterium content.



2g 4a

The d_5-glucose **2c** (275 mg) was next fed, as a mixture with 10.4 μCi of **2f,** to two 200-ml fermentations. Bioassay of the harvested broths indicated production of 370 mg of blasticidin and workup yielded 88 mg of pure recrystallized **4b.** A 1.07% incorporation of **2f** had been obtained, and ^{2}H NMR analysis (at 70°C) of a sample of **4b** revealed enrichments at δ 6.11 (H-6), 5.92 (H-7), 3.06 (N–CH_3), 2.70 (H-12), and 2.08 (H-14), as shown in Fig. 2c. The enrichments were 0.39, 0.56, 0.44, 0.15, and 0.28%, respectively. Thus, in the hexenuronic acid moiety the deuterium labels at C-2 and C-3 of glucose were retained, while those at C-6 were lost by oxidation, and the deuterium at C-4 was apparently lost during the oxidation/ transamination.



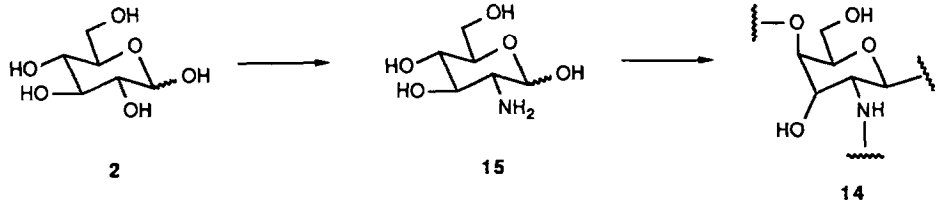
4b

Labeling of the *N*-methyl group can be explained in a straightforward manner (*37*) via glycolysis whereby C-6 and C-1 of glucose become C-3 of 3-phosphoglycerate. This becomes C-3 of serine, which becomes a methyl group via the tetrahydrofolate one-carbon pool. Deuterium at H-12 and H-14 can be explained via glycolysis and the Kreb's cycle which would provide [5-^{2}H]-α-ketoglutarate. Conversion (*38*) to [2,4-^{2}H]ornithine would involve [4*R*-^{2}H]NADPH generated from glycolysis of **2c,** and the labeled ornithine would be converted to [2,4-^{2}H]-β-arginine (*34, 35, 39*).

The apparent difference in deuterium enrichment at H-6 and H-7 (ratio 0.7 : 1.0) may be due simply to equilibration of phosphoglucose and phosphofructose by phosphoglucose isomerase, which shuttles a proton between C-1 and C-2 with less than 100% efficiency (*41*). More significant is the *retention* of both H-2 and H-3 of glucose at H-6 and H-7. This can be explained by a number of related pathways. Thus, as shown in Scheme 2, conversion of glucose to the 4-ketoglucoside **7** (R may be a nucleoside or—possibly—cytosine itself (*42*)) could be followed by Schiff base formation with pyridoxamine phosphate to give **8.** A 1,4-elimination of water (to give **9**) followed by hydration of the new imine would yield **10.** Cleavage to form pyridoxal phosphate could involve a concerted elimination of hydroxyl from C-2 (route a) or stepwise protonation at C-3 to **11** and then elimination of water involving the same hydrogen (route b). With either route, stereospecific reduction of the imine **12** would give the blasticidin moiety **13.**

Alternate pathways that would require loss of either H-2 or H-3 of glucose are excluded by our results. By way of example, route b of Scheme 2 with stepwise protonation to **10,** which has precedent in the formation of 3,6-dideoxyhexoses (*43*) and in the structures of blasticidin H (*44*) and the pentopyranines (*42*), could not involve subsequent elimination of the original glucose hydrogen (H-3). Initial formation of a 2-deoxyhexose via oxidation to a 3-keto sugar nucleoside by a pathway analogous to Scheme 2 (*15*) or by dehydration of a 4-ketohexose such as **6** is also excluded since this would remove the original H-3.

In a third example, **2c** was used to investigate the formation of the D-gulosamine moiety **14** of the ubiquitous antibiotic streptothricin F, **5** (*45*). In a formal sense,



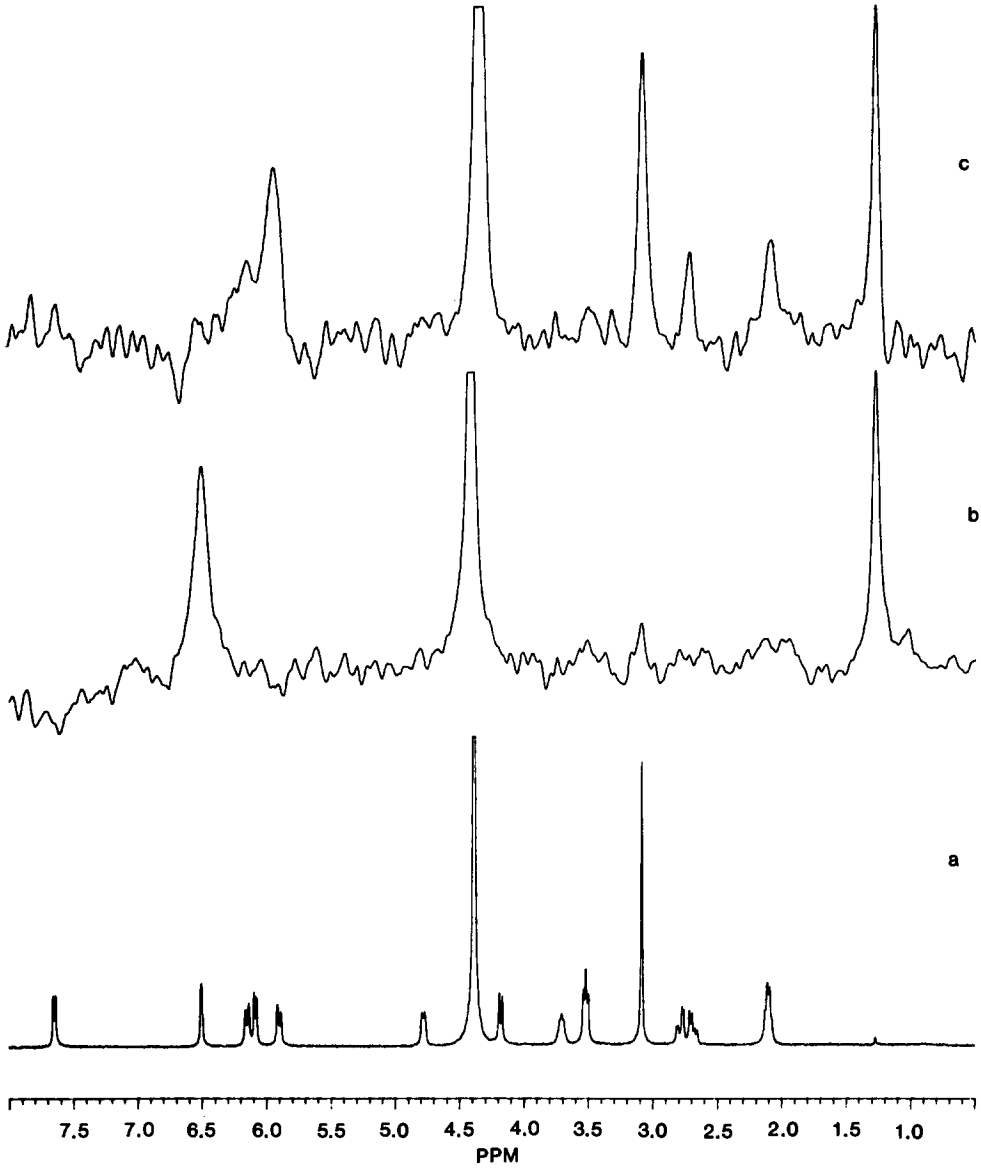
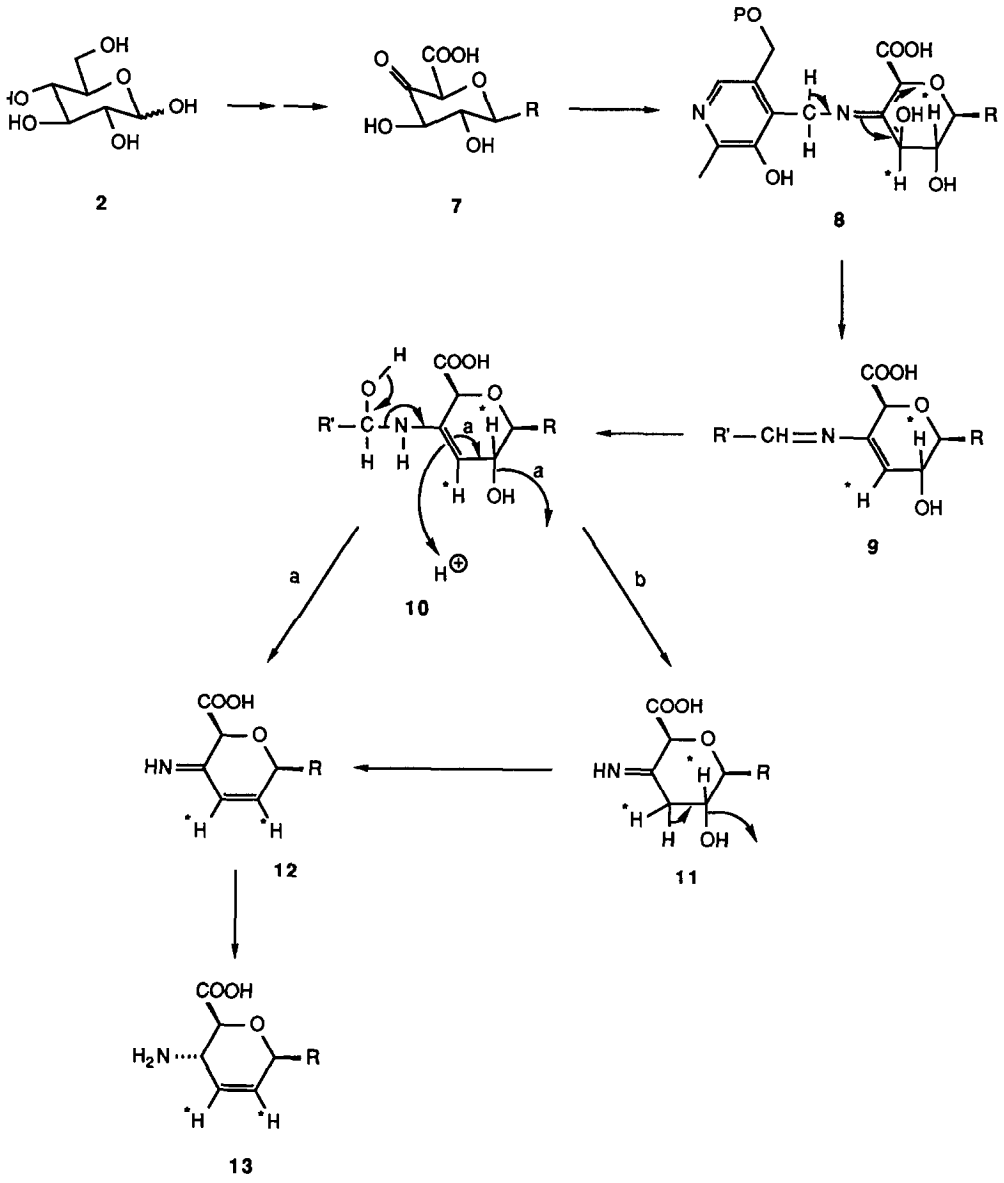


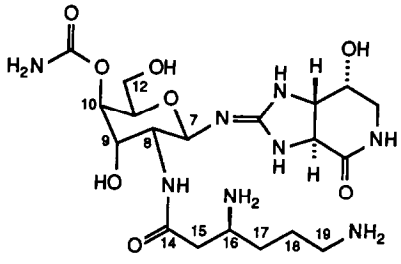
FIG. 2. (a) The 400-MHz ^{1}H NMR of authentic blasticidin S **4:** SW 6024 Hz, SI = TD 16K, AQ 1.36s, NS 48, PW 3.0, temp 60°C; (b) the 61.4-MHz ^{2}H NMR of **4a** from [1-^{2}H]glucose feeding: SW 952 Hz, SI 8K, TD 4K, AQ 2.15s, NS 23021, PW 13.5, LB 3Hz, Temp 60°C; (c) the 61.4-MHz ^{2}H NMR of **4b** from [2,3,4,6,6-$^{2}H_5$]glucose feeding: SW 807 Hz, SI 8K, TD 2K, AQ 1.268s, NS 38135, PW 6.0, LB 3Hz, temp 70°C.

conversion of **2** to **14** requires a transamination at C-2 and epimerizations at C-3 and C-4, in an unspecified order. Preliminary evidence with ^{14}C-labeled glucose and glucosamine, **15,** indicated that the latter was the probable first intermediate (*46, 47*). This was conclusively demonstrated by the intact incorporation of [2-^{13}C,^{15}N]-D-glucosamine (*48*).



SCHEME 2

A synthetic medium with glycerol and acetate as primary carbon sources was developed to minimize the extent to which glucose would label other portions of **5**. A mixture of **2c** (125 mg) and [1-^{14}C]-D-glucose, **2h** (23.6 μCi), was divided between two 250-ml production broths 24 h after inoculation with a seed culture (*45*) and the fermentations were worked up (*45*) 26 h later. A 1.8% incorporation based on radioactivity was obtained; this would have corresponded to a 4.2% enrichment in deuterium at each position. Regardless of the fate of the original labels at H-2, -3, and -4, the labels at H-6 (H-12 of **5**) were expected to be retained and provide an internal reference.



5

In the 41.4-MHz ^2H NMR spectrum of **5a,** aside from the resonance at δ 4.74 from residual deuterium in the solvent (deuterium-depleted water), the strongest signal was at δ 3.71 attributable to H-12. However, these were merely peaks emerging from a broad envelope from δ 3.1–5.5. In addition, a resonance at δ 1.74 was probably attributable to deuterium at H-17/H-18. The latter resonance indicated significant metabolism of **2c** into the β-lysine moiety, and probable metabolism into the streptolidine moiety (*vide infra*) would be responsible for part of the envelope (*49*). Thus, in the case of **5a,** it could not be unequivocally determined whether deuterium had been retained at H-8, -9, and/or -10 (streptothricin numbering) and in this case **2c** proved not to be a useful tool.

CONCLUSIONS

Since glucose is the ultimate primary organic precursor to the metabolites of microorganisms, plants, and animals, it is difficult to confine it to labeling only one part of a complex structure. In fact, with the use of [U-$^{13}C_6$]-D-glucose the hope is to find incorporation into all parts—albeit by specific rather than random pathways (*50*). In two of three pathways reported here, the inability to confine labeling from [2,3,4,6,6-$^2H_5$]-D-glucose, **2c,** to only the sugar moiety also proved impossible, but in only one case did this interfere with a clear interpretation of the relevant labeling pattern. Thus, it appears that **2c** can be used as a general tool for sorting out acceptable and unacceptable steps in any specific pathway of glucose modification. This would set the stage for subsequent work at the cell-free level to study individual steps, especially if they involve substrates unable to pass through cell walls and membranes (e.g., sugar nucleosides). Such work is in progress for blasticidin biosynthesis.

EXPERIMENTAL

General

^1H NMR spectra were taken on a Bruker AM 400 or HX 270 spectrometer; ^2H NMR spectra were obtained at 61.4 or at 41.4 MHz on a Bruker AM 400 or HX

270 spectrometer, respectively. ^2H NMR spectra were proton-decoupled and run unlocked. Samples were run in 5-mm tubes on the AM 400 spectrometer or in 0.5-ml cylindrical inserts for 10-mm tubes (HX 270). ^2H NMR samples were prepared in d_5-pyridine (sarubicin) or in deuterium-depleted water spiked with 25 μl *tert*-butanol for chemical shift reference (1.27 ppm) and deuterium quantitation (0.38 μmol) (blasticidin and streptothricin).

All radioactivity measurements were carried out using a Beckman LS 8000 or 7800 liquid scintillation counter; counting efficiencies with the LS 8000 were determined by spiking with *n*-[^{14}C]hexadecane standard purchased from Amersham/Searle while automatic quench correction with an external standard was used with the LS 7800.

Linear analytical thin-layer chromatography for sarubicin and blasticidin were carried out on precoated Kieselgel 60 F_{254} aluminum-backed sheets, and circular analytical TLC for streptothricin was done with 3.5 × 3.5-in. squares of the same material. Ion exchange resins were purchased from Bio-Rad, and Sephadex LH-20 was purchased from Sigma Chemical Co.

[2,3,4,6,6-$^2H_5$]-D-Glucose was purchased from MSD Isotopes (St. Louis, MO). [U-^{14}C]- and [1-^{14}C]-D-Glucose were purchased from New England Nuclear Corp. D_2O (99.8 atom%) and deuterium-depleted water were purchased from Aldrich Chemical Co.

Culture Conditions and Isolation

Sarubicin A. S. helicus (UC-5837) was maintained at 5°C in petri dishes on agar composed of 0.4% yeast extract, 1.0% malt extract, 0.4% dextrose, and 2.0% agar. Seed cultures were prepared by inoculating 100 ml of seed medium in a 500-ml Erlenmeyer flask; the seed medium was composed of 2.5% Pharmamedia, 2.5% dextrose in tap water and was adjusted to pH 7.2 with 1 N NaOH (*30*). The flasks were incubated at 32°C and 225 rpm on a Lab-line gyrotory incubator-shaker for 24 h. Production broths (250 ml in 1-liter Erlenmeyer flasks) consisting of 0.5% dextrose, 0.1% $(NH_4)_2SO_4$, 0.5% $CaCO_3$, and 0.1% trace salts solution were inoculated with 10 ml (4%, v/v) of the seed culture and incubated at 32°C, 225 rpm for 72 h. The trace salts stock was made up of 20% $MgSO_4 \cdot 7H_2O$, 5.0% $MnSO_4 \cdot H_2O$, 1.0% $ZnSO_4 \cdot H_2O$, 0.6% $FeSO_4 \cdot 7H_2O$, and 0.2% $CoCl_2 \cdot 6H_2O$ in deionized water.

After harvesting, the combined broths were adjusted to pH 3 (HCl), filtered, saturated with $(NH_4)_2SO_4$, and extracted with three portions of EtOAc. The extracts were dried (Na_2SO_4), filtered, concentrated, and chromatographed on silica gel. Elution with 10% MeOH/$CHCl_3$ gave pure sarubicin A.

Blasticidin S. The strain ATCC 21024 of *S. griseochromogenes* was maintained at 5°C on agar slants composed of 0.4% yeast extract, 1.0% malt extract, 0.4% dextrose, and 2% agar adjusted to pH 7.3. Seed cultures were prepared by inoculating 50 ml of seed medium, in a 250-ml Erlenmeyer flask, which was composed of 2.0% glucose, 1.0% beef extract, 1.0% polypeptone, and 0.2% NaCl in double-distilled water at pH 7.5. The Erlenmeyer flasks were incubated at 29°C and 225 rpm on a Lab-line gyrotory incubator–shaker for 48 h. Production broths (200 ml

in 1-liter baffled flasks, stock No. 2547, from Bellco Glass, Inc., Vineland, NJ) consisting of 5% sucrose, 1% soybean meal, 2.5% wheat embryo, 2.5% dried brewers yeast, and 0.6% NaCl in double-distilled water at pH 7.0 were inoculated with 4 ml of seed culture (2%, v/v) and incubated at 29°C, 226 rpm for 120 h in the gyrotory incubator–shaker.

After harvesting, the broth was centrifuged in an IEC Model B-20A centrifuge (9000 rpm, 11,000*g*) for 20 min. The pellets were washed with a minimum amount of water and centrifuged again, and the combined washings and supernatant were adjusted to pH 2.3 and allowed to stand for 30 min. The precipitated materials were removed by centrifugation, and the supernatant was adjusted to pH 7 and passed through a Bio-Rad AG2-X8 (OH^-, 100–200 mesh) column. The nonbound material and the water washings were simultaneously neutralized using 6 N HCl while collecting in order to avoid any decomposition of blasticidin S at very basic pH. The resulting yellowish brown solution was adjusted to pH 5.5 and loaded onto a Bio-Rad AG50W-X2 (H^+, 100–200 mesh) column, washed first with deionized water and then with 5% pyridine, and finally eluted with 1.2% NH_4OH. Fractions containing blasticidin S (checked by uv, and by TLC, silica gel, solvent *n*BuOH : MeOH : NH_3 : H_2O, 5 : 2 : 2 : 1) were collected, concentrated *in vacuo* to remove all NH_3, and lyophilized. The resulting solid was adjusted to ca. pH 6.8 and recrystallized from CO_2-free water : methanol to give pure blasticidin S as a white powder.

B. circulans (obtained from Department of Microbiology, Oregon State University) was used for the bioassay of blasticidin S. A stock spore suspension of *B. circulans* in 0.9% saline was prepared and stored at 4°C. For the bioassay, the stock spore suspension was diluted (1 : 500) in peptone agar (prepared by mixing 0.5 g of peptone in 100 ml of double-distilled H_2O at pH 9.0 with 1.5 g of agar) and 10 ml was placed on each petri plate. Paper disks (0.5-inch diam) were set on the agar (three per plate) and 75 μl of the test solution was placed on each disk. This was incubated at 37°C for 16 h and the inhibition zone was then measured. The log concentration vs (inhibition zone diameter)^2 normally gave a straight line graph and the concentration was measured from a standard curve prepared with authentic blasticidin S.

Streptothricin F. An improved strain of *S.* L-1689-23 was maintained at 5°C on agar slants composed of 1.0% malt extract, 0.4% yeast extract, 0.4% dextrose, and 2.0% agar, adjusted to pH 7.3. Seed cultures were prepared by inoculating 50–250 ml of medium containing 0.3% beef extract, 0.5% yeast extract, 0.5% tryptone, 0.1% dextrose, and 2.4% cornstarch. The cultures, contained in Erlenmeyer flasks of four to five times the broth volume, were incubated at 29°C and 200 rpm for 2.5 days. Production broths of O'Brien's medium (*51*) were modified to include $CaCO_3$ and additional carbon sources. O'Brien's medium composed of 0.2% glycine, 0.23% NaOAc · $3H_2O$, 0.02% K_2HPO_4, 0.05% $MgSO_4$ · $7H_2O$, 0.0016% $CuSO_4$ · $5H_2O$, 0.0013% $MnSO_4$ · $4H_2O$, 0.0025% $FeSO_4$ · $7H_2O$, 0.0066% $CaCl_2$ · $2H_2O$, and 0.003% $ZnSO_4$ · $7H_2O$ was autoclaved. The equivalent of 1.0% (w/v) of powdered $CaCO_3$ was autoclaved separately and then added to the broth. Finally 2.0% glycerol and 0.25% dextrose (w/v) were dissolved in a minimal

volume of distilled water and added by syringe in a sterile manner via a Millipore filter.

Cultures (250 ml) in 1-liter Erlenmeyer flasks were incubated at 29°C and 200 rpm for 50 h. These were worked up and bioassayed as previously described (*45*).

Labeling Experiments

Fermentation of S. helicus in the presence of D_2O. Two 200-ml production broths were prepared with deionized water (180 ml) and D_2O (20 ml). These were inoculated with a seed culture and incubated for 72 h. Workup (acidification with 6 N HCl, salting with 250 g $(NH_4)_2SO_4$, and extraction with 3 × 300 ml EtOAc) gave a crude product that was chromatographed on a column (5 × 23 cm) of silica gel 60 (230–400 mesh) to yield crude product that was recrystallized from chloroform, affording 19.0 mg of **3a.** ^2H NMR (61.4 MHz, d_5-pyridine) δ 1.3, 1.8, 2.7, 3.9, 4.2, 5.4, 7.2 (solvent), 7.6 (solvent), 8.8 (solvent).

*Incorporation of [2,3,4,6,6-$^2H_5$]-D-glucose (**2c**) into sarubicin A.* Two 200-ml production fermentations were incubated for 26.5 h, at which time a mixture of **2c** (135 mg) and [U-^{14}C]-D-glucose (**2f,** 22.6 μCi) in 2.0 ml of water was added in a sterile manner through a Millipore filter. After a total of 72 h of incubation, workup afforded 56.0 mg of pure **3b** (9.65×10^6 dpm/mmol, 3.7% incorporation). ^2H NMR (61.4 MHz, d_5-pyridine) δ 1.3, 1.8, 7.2 (solvent), 7.6 (solvent), 8.8 (solvent).

*Incorporation of [1-^2H]-D-glucose (**2g**) into blasticidin S.* Two 250-ml production fermentations in regular 1-liter Erlenmeyer flasks were incubated for 44 h, whereupon [1-^2H]-D-glucose (**2g,** 90.5 mg) and **2f** (18 μCi) in 10 ml of water were added in a sterile manner. After a total of 120 h, bioassay indicated 74 mg of blasticidin and workup through elution of the AG50W-X2 resin with 1.2% NH_4OH yielded 47 mg of impure **4a.** Recrystallization (3×) yielded 11 mg of pure product (1.77×10^6 dpm/mmol, 0.72% incorporation). ^2H NMR (61.4 MHz, deuterium-depleted water, 60°C) δ 1.28 (*t*-BuOH), 4.38 (HOD), 6.55.

*Incorporation of **2c** into blasticidin S.* Two 200-ml fermentations were carried out in 1-liter baffled flasks. After 44 h a mixture of **2c** (275 mg) and **2f** (10.4 μCi) was added in a sterile manner, and the fermentations were worked up after an additional 76 h. Bioassay of the initial broth indicated the presence of 370 mg of blasticidin S, and workup yielded 88 mg of **4b** after recrystallization (2.8×10^5 dpm/mmol, 1.07% incorporation). ^2H NMR (61.4 MHz, deuterium-depleted water, 70°C) δ 1.28 (*t*-BuOH), 2.08, 2.70, 3.06, 4.30 (HOD), 5.92, 6.11.

*Incorporation of **2c** into streptothricin F.* Two 250-ml fermentations were fed a mixture of **2c** (125 mg) and [1-^{14}C]-D-glucose (**2h,** 23.6 μCi) in a sterile manner 24 h after inoculation with a seed culture. After an additional 26 h the fermentation was stopped (bioassay indicated 183 mg of **5**), and workup yielded 224 mg of streptothricin F helianthate (3.14×10^6 dpm/mmol, 1.8% incorporation). A portion of this was converted to hydrochloride for NMR studies. ^2H NMR (41.4 MHz, deuterium-depleted water, pH 6.8, 10°C) δ 1.28 (*t*-BuOH), 1.74, broad envelope from 3.1 to 5.5 with identifiable peaks at 3.71 and 4.74.

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