

## Isolation and Identification of Derivatives Formed in the Course of Intracellular Accumulation of Thiogalactosides by *Escherichia coli*<sup>1</sup>

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Isolation of a derivative of isopropyl  $\beta$ -D-thiogalactopyranoside synthesized by a galactoside permease-possessing strain of *Escherichia coli* is described. The derivative is identified as the 6-O-acetyl ester. Several similarly modified thiogalactosides are identified as the acetyl esters of the respective thiogalactosides.

### INTRODUCTION

Rickenberg *et al.* (1) found that during the course of accumulation of radioactive methyl  $\beta$ -D-thiogalactoside (a nonhydrolyzable analog of lactose) by a strain of *Escherichia coli* possessing a galactoside permease, about 5% of the radioactivity which accumulated in the cells chromatographed with an  $R_f$  different from that of the original thiogalactoside. Later, we showed that all S<sup>35</sup>-containing  $\beta$ -D-thiogalactosides available (methyl, phenyl, isopropyl, and  $\beta$ -D-galactosyl) were chemically altered when accumulated by galactoside-permease-possessing strains and the newly formed compounds were elaborated into the medium.<sup>3</sup>

Studies by Zabin *et al.* (2) on the synthesis of derivatives of thiogalactosides by cell extracts suggest that an understanding of the mechanism of transport of sugars in bacterial cells may result from a study of the thiogalactoside derivatives and their synthesis.

In this communication we report the isolation and identification of isopropyl 6-O-acetyl- $\beta$ -D-thiogalactopyranoside (6-acetyl-

IPTG), as the derivative formed from isopropyl  $\beta$ -D-thiogalactopyranoside (IPTG) by strain ML 308.

### EXPERIMENTAL

#### BIOSYNTHESIS AND ISOLATION OF A MONOACETYLATED THIOGALACTOSIDE

To a young culture of *E. coli* strain ML 308 in 4 l. of medium 63 (3) supplemented with 0.4% sodium succinate was added 806 mg. (3.39 mmoles) of isopropyl  $\beta$ -D-thiogalactopyranoside-S<sup>35</sup> (IPTG) with a specific activity of  $3.55 \times 10^6$  counts/min./mmole. After 24 hr. incubation with aeration at 37°C., the bacteria were removed by centrifugation and the culture medium was passed through a column of Darco G-60-Celite-545 (1:1), 10 cm. long  $\times$  5 cm. wide. After the column was washed with 200 ml. water, a radioactive material was eluted with 80% ethanol in water. Chromatography in 1-propanol-water (3:1), followed by radioautography, revealed only one radioactive spot with an  $R_f$  of 0.81. IPTG has an  $R_f$  in this solvent of 0.71.

The column eluate was dried to yield 648 mg. of a semicrystalline, brown solid. This was recrystallized thrice from ethyl acetate, using charcoal to decolorize, to a constant specific activity of 1320 counts/min./mg. (corrected for decay of S<sup>35</sup>). On the basis of a molecular weight of 280, this corresponds to  $3.7 \times 10^6$  counts/min./mmole. This material had a melting point of 129-130°C. (uncorr.)

*Anal.* Calcd. for C<sub>11</sub>H<sub>20</sub>O<sub>6</sub>S (280.34): C, 47.12; H, 7.19; S, 11.44. Found C, 47.18; H, 7.29; S, 11.39.

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IDENTIFICATION OF ISOPROPYL 6-O-ACETYL- $\beta$ -D-THIOGALACTOPYRANOSIDE

The isolated compound was reacted with 0.5 *N* KOH at 37°C. for 14 hr. The solution was then neutralized with HCl and chromatographed in 1-propanol-water (3:1). Radioautography showed that all (greater than 98%) of the radioactivity was associated with a single spot coincident in position with IPTG.

An estimation of the amount of IPTG in another aliquot of the alkali-treated solution was made by measurement of inducing activity for  $\beta$ -galactosidase in *E. coli* ML 3 (3). The unhydrolyzed derivative has no inducing activity. The differential rate of synthesis of  $\beta$ -galactosidase for  $2 \times 10^{-4}$  *M* hydrolyzed derivative (based on a molecular weight of 280) was 2840 (increase in units of enzyme per increase in mg. dry weight of bacteria) compared to 3600 for  $2 \times 10^{-4}$  *M* IPTG under the same conditions. This corresponds to a concentration of IPTG in the hydrolyzate of  $1.7 \pm 0.2 \times 10^{-4}$  *M*.

Reaction of the derivative with hydroxylamine in basic solution, acidification, and addition of ferric chloride gave a reddish colored solution, suggesting that the derivative was an ester. The hydroxamic acid was identified as acethydroxamic acid by chromatography. It had an  $R_f$  of 0.70 in 1-propanol-water (3:1), and the position was coincident with that of known acethydroxamic acid.

A quantitative estimation of acethydroxamate by the method of Hestrin (4) gave 0.99 mole acetate per atom of sulfur. Thus the identification of the derivative has been made as a monoacetylated isopropyl  $\beta$ -D-thiogalactoside (acetyl-IPTG).

The position of the ester linkage was determined by means of a periodate oxidation. Acetyl-IPTG (240 mg.) was dissolved in 10 ml. of approximately 0.022 *M* sodium metaperiodate. After 8 hr. at room

temperature, 1-ml. aliquots were titrated potentiometrically to pH 7.0 with 0.82 and 0.84 ml. of 0.0102 *N* sodium hydroxide, or 0.99 mole acid were produced per mole of the acetyl-IPTG. This places the acetyl group on position 6 of the galactose moiety, as occupancy of any of the other potentially free positions (2, 3, or 4) would not have yielded acid on periodate oxidation.

Small quantities of similarly bacterial-synthesized derivatives of methyl, phenyl, and  $\beta$ -D-galactosyl- $\beta$ -D-thiogalactosides were isolated chromatographically and shown to react with hydroxylamine in basic solution to yield acethydroxamate and the original thiogalactoside. The methyl and phenyl thiogalactosides both yielded one mole of acethydroxamate per sulfur atom while the thiogalactoside ( $\beta$ -D-galactosyl- $\beta$ -D-thiogalactoside) was converted both to a mono and a diacetyl derivative indicating that both galactose moieties could be acetylated.

Presumably because of the oxidation of the sulfur atom in the molecule, estimates of periodate uptake did not lead to consistent values. Periodate uptake continued long after acid production was complete. At 8 hr. 2.58 moles periodate had disappeared per mole of acid produced. This observation is entirely consistent with those of Bonner and Drisko (5) on periodate oxidation of thioglycosides.

## REFERENCES

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