Notes

PREPARATION OF LABELED AKLANONIC ACID AND ITS BIOCONVERSION TO ANTHRACYCLINONES BY MUTANTS OF *STREPTOMYCES GRISEUS*

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In a previous communication we reported on the isolation and chemical structure of aklanonic acid, a new anthraquinone derivative produced by a *Streptomyces* strain[1]. On the basis of structural considerations we suggested that this compound might be an intermediate at an early stage in the biosynthetic pathway of anthracyclines. This idea was supported by the observation that aklanonic acid when supplemented to cultures of blocked mutants of *Streptomyces griseus* incapable of producing anthracyclinones induced the formation of ε-rhodomycinone, 7-deoxy-ε-rhodomycinone, daunomycinone, and anthracyclinone 1P/II, respectively[2]. The present study reports on the preparation of radioactively labeled aklanonic acid and its use in feeding experiments in order to confirm that the ring skeleton of aklanonic acid is indeed transformed to the ring systems of ε-rhodomycinone and the recently described anthracyclinone 1P/II (7-hydroxy-bisanhydro-ε-rhodomycinone).

[*U*-^{14}C]Aklanonic acid was prepared as follows: The new aklanonic acid accumulating mutant strain NTG 061 derived from *Streptomyces galilaeus* F 198 was used for fermentation. Culture conditions and production media were as described previously[1]. After 24 hours incubation the cultures were supplemented with D-[*U*-^{14}C]glucose (purchased from UVVVR Prague, Czechoslovakia; total radioactivity added to the cultures: 440×10^6 dpm) and incubated for 2 more days. The isolation of [^{14}C]aklanonic acid was performed as described for the unlabeled compound. The mycelium harvested from 450 ml of fermentation broth yielded a total of 61 mg of [*U*-^{14}C]aklanonic acid with a specific activity of 77,463 dpm/mg.

Feeding Experiments

The characteristics of the mutant strains used (O_1P_7 and $1P_5$) which were completely blocked in the production of anthracyclines and anthracyclinones, and the fermentation conditions employed have been described elsewhere. [*U*-^{14}C]Aklanonic acid (3.0 mg in 3 ml of MeOH) was added to each of five 500-ml flasks containing 80 ml of a 24-hour old culture of strain O_1P_7 (total radioactivity of aklanonic acid 15 mg= 1,161,945 dpm). After further incubation for 24 hours the red pigments were extracted from both the mycelium and culture filtrate with acetone and EtOAc, respectively. After evaporation to dryness the residues were redissolved in 20 ml $CHCl_3$. The solution was dried over Na_2SO_4 and then concentrated to about 3 ml. After addition of 0.5 ml acetone the mixture was chromatographed on KH_2PO_4-buffered silica gel by use of the $CHCl_3$-acetone, 10: 2 system. ε-Rhodomycinone was eluted as the main band followed by two yellow bands consisting of small amounts of unchanged aklanonic acid and an unidentified compound. 7-Deoxy-ε-rhodo-

Fig. 1. Chemical structure of aklanonic acid.

Table 1. Bioconversion of [*U*-^{14}C]aklanonic acid to ε-rhodomycinone and compound 1P/II (7-hydroxy-bisanhydro-ε-rhodomycinone) by two mutants of *Streptomyces griseus* blocked in the biosynthesis of anthracyclinones and anthracyclines.

Converting strain	Labeled product isolated	Specific activities (dpm/mg)	Conversion (%) $\left(\frac{\text{Bq/mmol product} \times 100}{\text{Bq/mmol aklanonic acid}}\right)$
O_1P_7	ε-Rhodomycinone	74,520	104 (±7)
$1P_5$	Compound 1P/II	75,249	100 (±7)

mycinone (first minor band) and daunomycinone glycosides which remained at the starting point[2] were not isolated. Yield: 2.5 mg of pure [*U*-^{14}C]-ε-rhodomycinone.

Cultivation of strain $1P_5$ with labeled aklanonic acid was carried out as described for strain O_1P_7. The culture broth of five flasks was centrifuged and the mycelium extracted twice with 20 ml of acetone. The resulting red extracts were combined, diluted with H_2O and then reextracted with $CHCl_3$. The red chloroform layer was dried with Na_2SO_4 and evaporated to dryness. The residue was redissolved in a small amount of benzene and purified by column chromatography on oxalic acid-buffered silica gel. Elution was with benzene. From the main red zone 0.5 mg of [*U*-^{14}C]-7-hydroxy-bisanhydro-ε-rhodomycinone were obtained. Thin-layer chromatography: Precoated aluminium sheets (E. Merck Co.) silica gel 60 F_{254} impregnated with 0.5 N oxalic acid were used. Rf values ($CHCl_3$ - acetone, 10: 2): aklanonic acid 0.39, ε-rhodomycinone 0.58, compound 1P/II 0.75.

Results and Discussion

Feeding of D-[*U*-^{14}C]glucose to cultures of the aklanonic acid-producing mutant strain NTG 061 resulted in the formation of [*U*-^{14}C]aklanonic acid with the specific activity of 77,463 dpm/mg. The incorporation efficiency of ^{14}C from D-[*U*-^{14}C]glucose into aklanonic acid was ~1.05%.

When radiolabeled aklanonic acid was supplemented to fermentations of the blocked mutant strain O_1P_7 the formation of several anthracyclinones was induced. The results were similar to those obtained with unlabeled material with respect to the number and chromatographic mobilities of the produced components. The main component ε-rhodomycinone was isolated and measured for radioactivity.

Cultures of the negative mutant strain $1P_5$ converted [*U*-^{14}C]aklanonic acid to several radioactive products. The red and blue bands in the chromatogram of the mixture corresponded to the zones found in the autoradiograph indicating that all produced pigments were biosynthesized by converting aklanonic acid. The major component 1P/II was isolated by column chromatography and measured for radioactivity.

Both radioactive products 1P/II and ε-rhodomycinone were identified by comparing their chromatographic mobilities and mass spectra with those of authentic materials. The chromatograms revealed that the purified conversion products were free of unchanged [^{14}C]-alkanonic acid. The specific activities of the compounds and percentage of conversion are listed in Table 1 indicating that the radioactivity of [^{14}C]aklanonic acid was completely incorporated into ε-rhodomycinone and 1P/II. These findings confirm that both anthracyclinones were indeed produced by bioconversion of intact aklanonic acid and it can be concluded that aklanonic acid is one of the first cyclic intermediates in the biosynthetic sequence to anthracyclinones and anthracyclines produced by *S. griseus*. Preliminary results show that aklanonic acid is similarly converted by other anthracycline-producing *Streptomyces* species.

References

1) ECKARDT, K.; D. TRESSELT, G. SCHUMANN, W. IHN & CH. WAGNER: Isolation and chemical structure of aklanonic acid, an early intermediate in the biosynthesis of anthracyclines. J. Antibiotics 38: 1034~1039, 1985

2) WAGNER, CH.; K. ECKARDT, G. SCHUMANN, W. IHN & D. TRESSELT: Microbial transformation of aklanonic acid, a potential early intermediate in the biosynthesis of anthracyclines. J. Antibiotics 37: 691~692, 1984