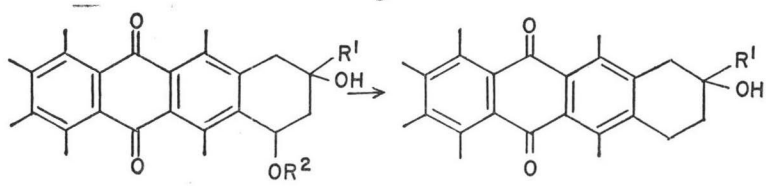
MICROBIAL CONVERSION OF ANTHRACYCLINE ANTIBIOTICS

Sir:

Almost all of the anthracycline antibiotics are active against tumor cells[1,2]. Some of them, such as adriamycin[3], are of great interest as clinical candidates for the treatment of various cancers. Because of the general antitumor activities of such antibiotics, it would be of interest to discover new ones. As a means of doing this we have attempted conversion of several anthracyclines to new agents by microbial means. In the course of this work we have found that certain facultative organisms grown anaerobically can bring about what is formally at least a benzylic hydrogenolysis of anthracycline antibiotics. The reaction is depicted schematically in Fig. 1.

Fig. 1.



R^1 = Alkyl or acyl, R^2 = H or Glycosyl

A mixture of organisms was isolated from sewage and grown in a medium consisting of PAS inorganic salts[4] supplemented with 0.1 % yeast extract, 0.1 % nutrient broth, and 0.005 % anthracycline substrate. The cells were cultured for 5~7 days at 25°C in sealed reagent bottles with no air-space remaining following inoculation. Neither reducing agents nor molecular oxygen destroying compounds were added. Maximum conversion was reached after five days. The final pH of the fermentations was 6.7~7.0.

Organisms identified from the original mixture were *Aeromonas hydrophila*, *Escherichia coli*, and *Citrobacter freundii*. It was found that each of the three organisms alone would cause the reductive cleavage. However, when the organisms were incubated in the presence of an adequate supply of oxygen and with shaking, reduction failed to occur. In the presence of DPNH dialyzed sonic extracts of an anaerobic culture of *A. hydrophila* also brought about the conversion.

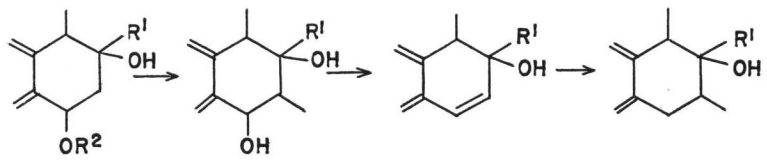
The products derived from the anthracycline substrates and unchanged anthracycline were isolated by extraction with various solvents, usually methylene chloride or ethyl acetate, at a pH of 8.6 in the cases of the basic antibiotics and at natural pH in the cases of the neutral substrates. The crude isolated materials were analyzed with TLC using silica on glass plates and the solvent systems chloroform - methanol (95 : 5), cyclohexane - ethyl acetate - 95 % ethanol (50 : 30 : 20), and chloroform - methanol - water (78 : 20 : 2). The extracted material was purified by silica gel chromatography in an appropriate solvent system followed by crystallization when possible. The pure 7-deoxyanthracycline aglycones were all known compounds and were characterized by comparison with authentic samples or with literature data. The criteria used for comparison were TLC, melting point, and ultraviolet, infrared, nuclear magnetic resonance and mass spectra.

The anthracycline antibiotics subjected to microbial conversion were steffimycin[5], steffimycin B[6], nogalamycin[7], and daunomycin[8]. In addition the aglycone of steffimycin, steffimycinone, was used. The reduction product of steffimycin, steffimycin B, and steffimycinone was 7-deoxysteffimycinone which had been obtained chemically from steffimycin. The deoxyaglycone was isolated, purified, and identified as the pure material when derived biologically from steffimycin and steffimycinone. Identification of 7-deoxysteffimycinone as a conversion product of steffimycin B was done by TLC on the fermentation mixture. Nogalamycin and daunomycin were converted to 7-deoxynogalarol[9], previously obtained chemically from nogalamycin, and 7-deoxydauno-

mycinone[10], respectively. Steffimycin was the only compound subjected to all modes of biological transformation, mixed sewage organisms, *A. hydrophila*, *E. coli*, *C. freundii*, and cell-free extracts from *A. hydrophila*. The principal product was 7-deoxysteffimycinone in each case although small amounts of other unidentified products were formed. Steffimycinone was subjected to the action of both cells and cell-free extract. In both cases the product was 7-deoxysteffimycinone which was identified in the cell fermentation by TLC on the mixture but was isolated from the cell-free medium and identified as the pure material.

In considering such an overall reductive cleavage several pathways seem possible. *A. priori* the most likely route would appear to be hydrolysis to aglycone and sugar followed by aglycone dehydration to a $\Delta^{7,8}$ system with subsequent reduction as indicated in Fig. 2.

Fig. 2.



A second possibility is hydrolysis as in Fig. 2 followed by a benzylic reductive cleavage as indicated in Fig. 1 (R^2=H). A third possibility would be a reductive cleavage without prior hydrolysis (Fig. 1, R^2=glycosyl). Other possibilities can be envisioned, but we regard them as being too unlikely to consider. Steffimycin recovery, which occurred in all cases and was as high as 61 %, and the yield of 7-deoxysteffimycinone (maximum for recrystallized material 47 %) were dependent on the biological system used and on the duration of the fermentation. No evidence has been found for the formation of the aglycone, steffimycinone, in those experiments involving steffimycin. In an experiment designed to establish whether or not steffimycinone was formed, a cell-free crude enzyme preparation was used to catalyze the conversion of 1 g of steffimycin. Extraction and chromatographic purification gave 7-deoxysteffimycinone, recovered steffimycin, and several minor unidentified fractions. Comparison of the unidentified materials with steffimycinone by TLC in the solvent systems already mentioned, showed no indication of the presence of steffimycinone. Since steffimycinone conversion to 7-deoxysteffimycinone was shown to occur at a rate less than half that observed when steffimycin was the reaction substrate, steffimycinone should be observed, if it were an intermediate, in an equilibrium mixture of substrate and product. The above findings concerning conversion products coupled with the demonstrated requirement for DPNH support the third possibility (reductive cleavage) as the most likely route.

So far as we are aware this is the first example of such a biotransformation being brought about by microorganisms although reductive cleavage of hydroxyl groups and α-hydroxy acids is well-known[11,12]. BACHUR, *et al*[13,14] and ASBELL and coworkers[15] have apparently found a similar type of reaction, but requiring TPNH instead of DPNH, in mammalian systems in which daunomycin and adriamycin are converted to 7-deoxyaglycones as well as other products[14,15]. A similar interpretation of the reaction pathway was advanced. It seems quite possible that the benzylic reduction reported here is much more general and could be carried out with a great many compounds, both antibiotics and non-antibiotics.

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