equivalent to 1667 units.

Tetracycline

The **tetracyclines** are four-ring molecules with five different sites for substitution (Fig. 24.9). These are **broad-spectrum bacteriostatic antibiotics** that inhibit synthesis of proteins by binding to the small ribosomal subunit in a manner that prevents aminoacyl transfer RNA from entering the acceptor sites on the ribosome. **Chlortetracycline**, the first antibiotic of this group, was isolated from cultures of *Streptomyces aureofaciens* in 1945. Recent studies in molecular biology of this mold have shown that over 300 genes are involved, and at least 72 separate enzymatic steps are involved in the synthesis of tetracycline. It is a secondary metabolite.

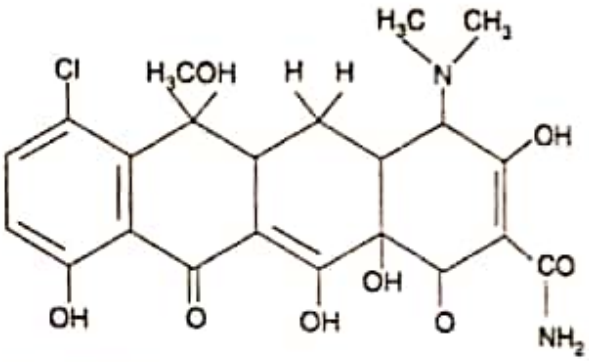

Fig. 24.9: Four-ring structure of chlortetracycline (aureomycin).

The basic structure of the tetracyclines consists of a napthacene ring system The production of tetracyclines is carried out in stirred fermentors with volumes up to 150,000 litres. The Fig. 24.10 illustrates the production process of chlortetracycline. *S. aureofaciens* inoculum is prepared by growing the organism at 28°C on agar plates of the following constitution: meat extract (2%), asparagine (0.05%), glucose (1%), K_2HPO_4 (0.5%) and agar (1.3%).

Inoculation of the shake flasks having the corn steep liquor broth (composition: corn steep liquor—2%, sucrose—3%, $CaCO_3$—0.5%; pH 5.2–6.2) is done where the spores are used as inoculum, incubated for 24 hours for allowing the organism to grow.

Inoculation of the prefermentor: Inoculum from the shake flasks is inoculated into the prefermentors, having the broth of the same composition as used in the shake flasks and incubated for 19–24 hours.

Fermentation: The constituents of the prefermentor are transferred to the fermentor consisting of sucrose (1%), cornsteep liquor (0.2%), $(NH_4)_2HPO_4$ (0.2%), $CaCO_3$ (0.1%), $MgSO_4.7H_20$ (0.025%), $ZnSO_4.7H_2O$

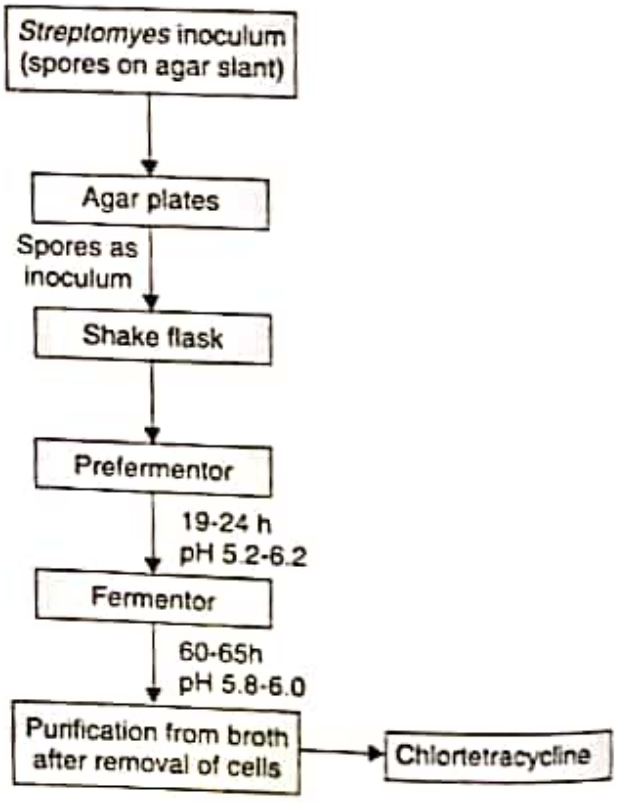

Fig. 24.10: Steps in production of chlortetracycline—*Streptomyces aureofaciens* in cornsteep liquor broth.

(0.005%), $CuSO_4.5H_2O$ (0.00033%), $MnCl_2.4H_20$ of pH 5.8–6.0 and incubated for 60–65 hours at 28°C for the production of chlortetracycline, a secondary metabolite into broth by *Streptomyces*. Glucose and phosphate are never to be added into the fermentation broth since repression of chlortetracycline synthesis occurs by both, thus severely reducing the yield of the antibiotic. Bacterial biomass is filtered and chlortetracycline is later purified from the filtrate.