Andersson et al. (1989) J. Biol. Chem. 264(14) 8222-8229.

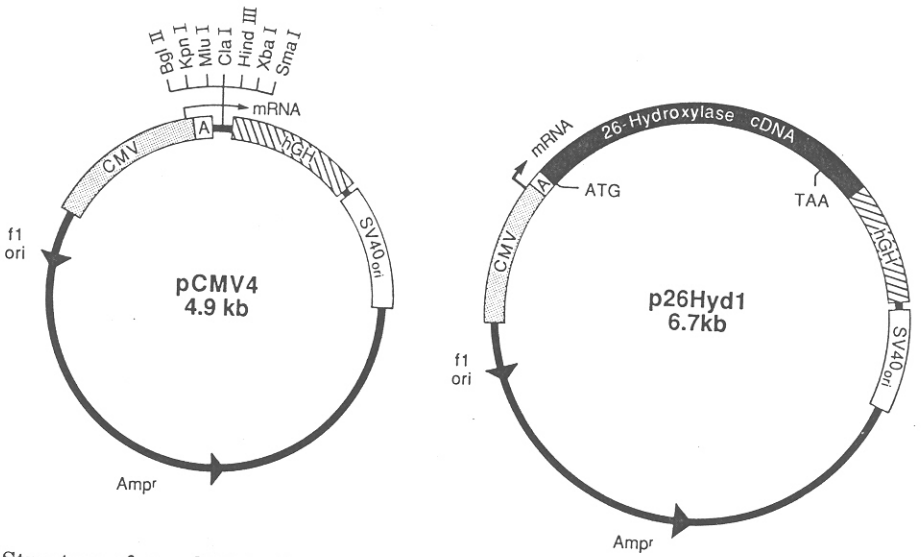

FIG. 2. **Structure of sterol 26-hydroxylase expression vector.** Plasmid pCMV4 represents the starting expression vector and contains the immediate early promoter region of the human cytomegalovirus (*CMV, stippled block*), a DNA copy of a segment of the alfalfa mosaic virus 4 RNA that contains a translational enhancer (*A*), a polylinker containing unique sites for the indicated restriction enzymes, transcription termination and polyadenylation signals from the human growth hormone gene (*hGH, hatched block*), and the SV40 origin of DNA replication and early region enhancer sequences ($SV40_{ori}$, *white block*). This plasmid also contains an *E. coli* gene encoding ampicillin resistance (*Amp*^{r}) and a bacteriophage f1 origin of DNA replication (*f1*). Plasmid p26Hyd1 contains a fragment of the rabbit sterol 26-hydroxylase cDNA corresponding to nucleotides 14–1852 of Fig. 1 cloned into the *Bgl*II and *Sma*I sites of the polylinker region of pCMV4 (see "Experimental Procedures"). The approximate positions of the initiator methionine (*ATG*) and translation termination (*TAA*) codons are indicated *below* the 26-hydroxylase cDNA insert.

TABLE II

Expression of sterol 26-hydroxylase cDNA in mammalian COS cells

Plasmid[a]	Total conversion[b]		Product/protein[c]	
	26-OH	26-COOH	26-OH	26-COOH
	%		*nmol/mg*	
Vector (pCMV4)	<1	<1	<0.2	<0.2
Adrenodoxin (pBADX-4)	<1	<1	<0.2	<0.2
P-450 reductase (pP450Red)	<1	<1	<0.2	<0.2
26-Hydroxylase (p26Hyd1)	21	<1	5.3	<0.2
26-Hydroxylase + P-450 reductase	24	<1	5.6	<0.2
26-Hydroxylase + adrenodoxin	18	76	4.3	18.2

[a] Approximately 2.2 μg of each of the indicated plasmids were transfected into duplicate dishes of COS-M6 cells as described under "Experimental Procedures."

[b] Conversion of 5β-[7β-^{3}H]cholestane-3α,7α,12α-triol into 5β-[7β-^{3}H]cholestane-3α,7α,12α,26-tetrol (26-OH) or 3α,7α,12α-trihydroxy-5β-[7β-^{3}H]cholestanoic acid (26-COOH) was measured 72 h after transfection by thin layer chromatography as described under "Experimental Procedures." The results represent the averages obtained from duplicate transfected dishes.

[c] Total cellular protein was estimated by the method of Bradford (51) and averaged 0.2 mg/60-mm dish.

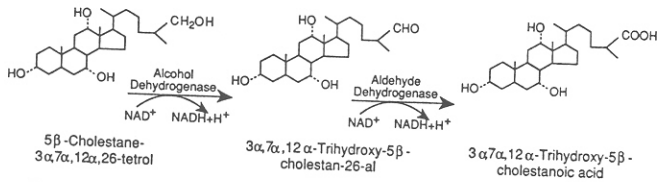

FIG. 3. **Proposed scheme for the oxidation of the side chain of 5β-cholestane-3α,7α,12α,26-tetrol by COS-M6 cells.** The substrates, products, and the enzymes and cofactors thought to be involved are indicated.

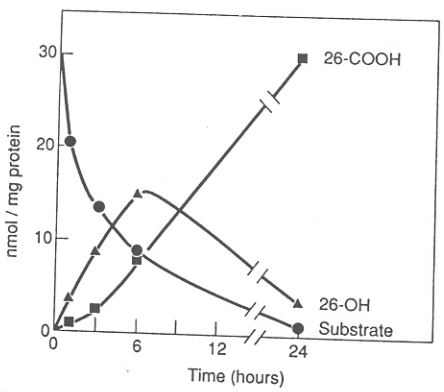

FIG. 4. **Time course for the conversion of 5β[7β-^{3}H]cholestane-3α,7α,12α-triol into 5β-[7β-^{3}H]cholestane-3α,7α,12α, 26-tetrol (*26-OH*) and 3α,7α,12α-trihydroxy-5β-[7β-^{3}H] cholestanoic acid (*26-COOH*) by COS-M6 cells.** Cells were co-transfected with plasmids encoding sterol 26-hydroxylase and adrenodoxin as described under "Experimental Procedures." On day 3 of the standard protocol, the medium was made 2.5 μM with 5β-[7β-^{3}H] cholestane-3α,7α,12α-triol. At the indicated times after addition, the medium in triplicate dishes was removed for thin layer and high performance liquid chromatography analyses, and the cells were harvested for protein determination.

rabbit genome. In this respect, the rabbit 26-hydroxylase is different from many of the characterized rat microsomal P-450 enzymes that are encoded by multigene families (33).

DISCUSSION

In the present paper, we describe the cloning, structure, and expression of a rabbit liver mitochondrial sterol 26-hydroxylase cDNA. The sequence of this enzyme reveals it to be a member of the cytochrome P-450 superfamily of mixed function monooxygenases. When expressed in cultured cells, the cDNA directs the synthesis of an enzyme that faithfully