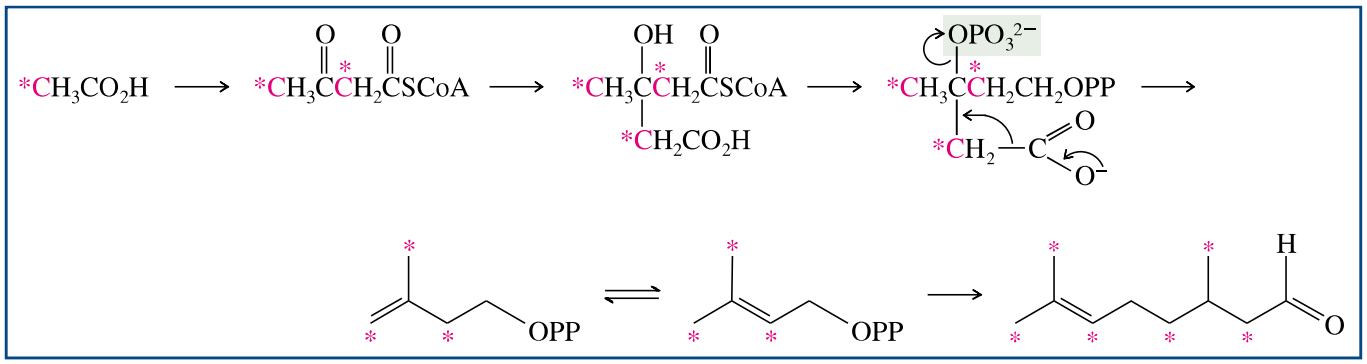


FIGURE 26.8 Diagram showing the distribution of the ^{14}C label (*C) in citronellal biosynthesized from acetate in which the methyl carbon was isotopically enriched with ^{14}C.

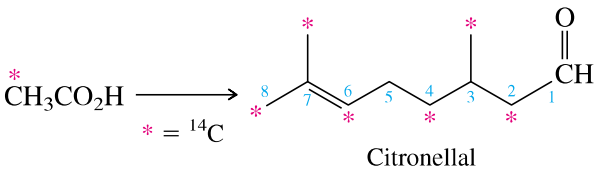


Figure 26.8 traces the ^{14}C label from its origin in acetic acid to its experimentally determined distribution in citronellal.

PROBLEM 26.11 How many carbon atoms of citronellal would be radioactively labeled if the acetic acid used in the experiment were enriched with ^{14}C at C-1 instead of at C-2? Identify these carbon atoms.

A more recent experimental technique employs ^{13}C as the isotopic label. Instead of locating the position of a ^{14}C label by a laborious degradation procedure, the ^{13}C NMR spectrum of the natural product is recorded. The signals for the carbons that are enriched in ^{13}C are far more intense than those corresponding to carbons in which ^{13}C is present only at the natural abundance level.

Isotope incorporation experiments have demonstrated the essential correctness of the scheme presented in this and preceding sections for terpene biosynthesis. Considerable effort has been expended toward its detailed elaboration because of the common biosynthetic origin of terpenes and another class of acetate-derived natural products, the steroids.

26.11 STEROIDS: CHOLESTEROL

Cholesterol is the central compound in any discussion of steroids. Its name is a combination of the Greek words for "bile" *(chole)* and "solid" *(stereos)* preceding the characteristic alcohol suffix *-ol*. It is the most abundant steroid present in humans and the most important one as well, since all other steroids arise from it. An average adult has over 200 g of cholesterol; it is found in almost all body tissues, with relatively large amounts present in the brain and spinal cord and in gallstones. Cholesterol is the chief constituent of the plaque that builds up on the walls of arteries in atherosclerosis.

Cholesterol was isolated in the eighteenth century, but its structure is so complex that its correct constitution was not determined until 1932 and its stereochemistry not

verified until 1955. Steroids are characterized by the tetracyclic ring system shown in Figure 26.9*a*. As shown in Figure 26.9*b*, cholesterol contains this tetracyclic skeleton modified to include an alcohol function at C-3, a double bond at C-5, methyl groups at C-10 and C-13, and a C_8H_{17} side chain at C-17. Isoprene units may be discerned in various portions of the cholesterol molecule, but the overall correspondence with the isoprene rule is far from perfect. Indeed, cholesterol has only 27 carbon atoms, three too few for it to be classed as a triterpene.

Animals accumulate cholesterol from their diet, but are also able to biosynthesize it from acetate. The pioneering work that identified the key intermediates in the complicated pathway of cholesterol biosynthesis was carried out by Konrad Bloch (Harvard) and Feodor Lynen (Munich), corecipients of the 1964 Nobel Prize for physiology or medicine. An important discovery was that the triterpene *squalene* (see Figure 26.6) is an intermediate in the formation of cholesterol from acetate. Thus, *the early stages of cholesterol biosynthesis are the same as those of terpene biosynthesis* described in Sections 26.8–26.10. In fact, a significant fraction of our knowledge of terpene biosynthesis is a direct result of experiments carried out in the area of steroid biosynthesis.

How does the tetracyclic steroid cholesterol arise from the acyclic triterpene squalene? Figure 26.10 outlines the stages involved. It has been shown that the first step is oxidation of squalene to the corresponding 2,3-epoxide. Enzyme-catalyzed ring opening of this epoxide in step 2 is accompanied by a cyclization reaction, in which the electrons of four of the five double bonds of squalene 2,3-epoxide are used to close the A, B, C, and D rings of the potential steroid skeleton. The carbocation that results from the cyclization reaction of step 2 is then converted to a triterpene known as *lanosterol* by the rearrangement shown in step 3. Step 4 of Figure 26.10 simply indicates the structural changes that remain to be accomplished in the transformation of lanosterol to cholesterol.

Lanosterol is one component of lanolin, a mixture of many substances that coats the wool of sheep.

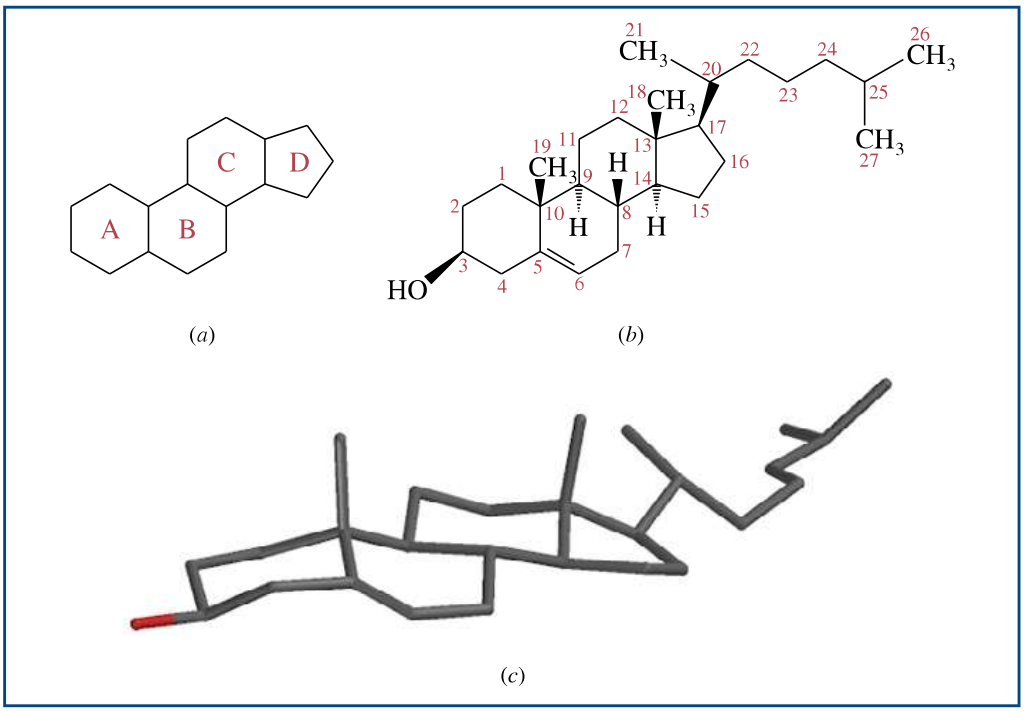
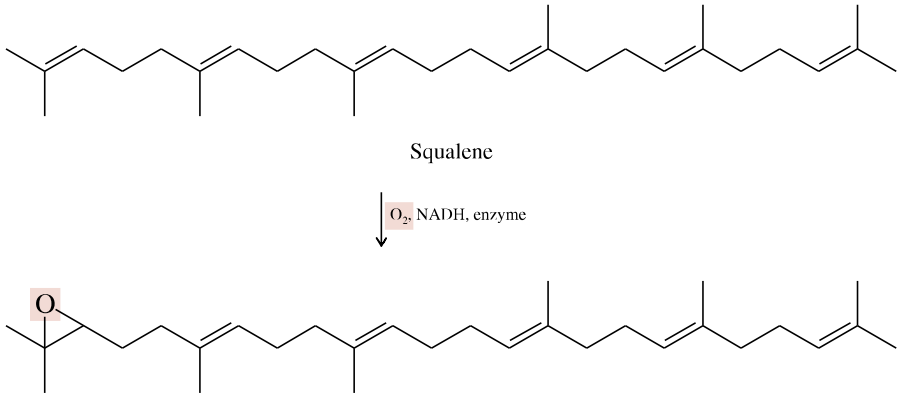
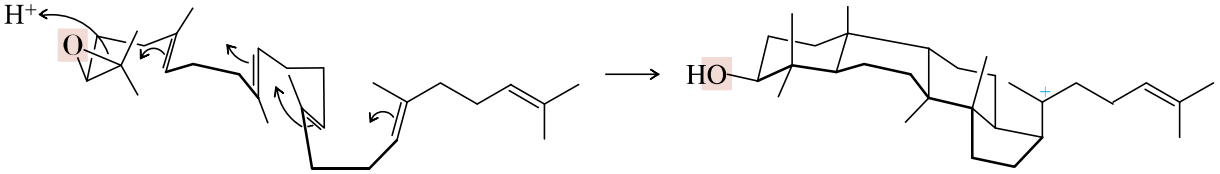


FIGURE 26.9 (*a*) The tetracyclic ring system characteristic of steroids. The rings are designated A, B, C, and D as shown. (*b*) and (*c*) The structure of cholesterol. A unique numbering system is used for steroids and is indicated in the structural formula.

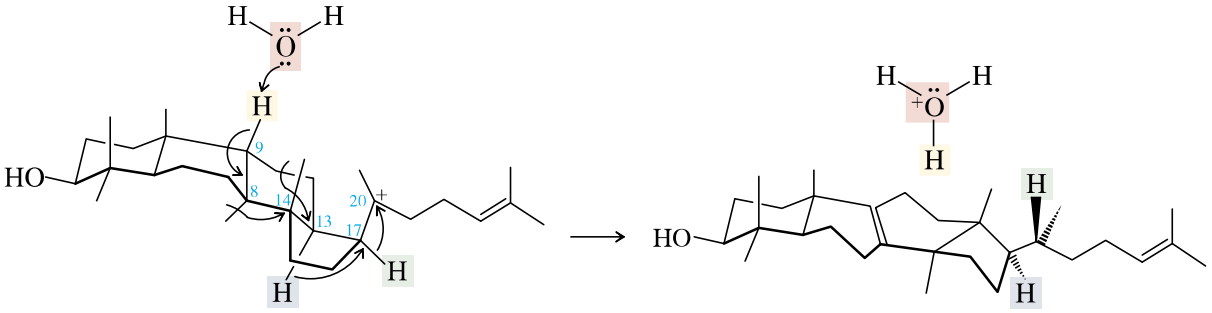
Step 1: Squalene undergoes enzymic oxidation to the 2,3-epoxide. This reaction has been described earlier, in Section 16.14.



Step 2: Cyclization of squalene 2,3-epoxide, shown in its coiled form, is triggered by ring opening of the epoxide. Cleavage of the carbon–oxygen bond is assisted by protonation of oxygen and by nucleophilic participation of the π electrons of the neighboring double bond. A series of ring closures leads to the tetracyclic carbocation shown.



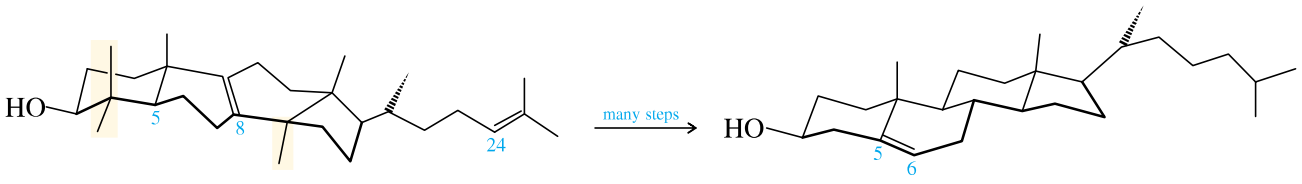
Step 3: Rearrangement of the tertiary carbocation formed by cyclization produces lanosterol. Two hydride shifts, from C-17 to C-20 and from C-13 to C-17, are accompanied by methyl shifts from C-14 to C-13 and from C-8 to C-14. A double bond is formed at C-8 by loss of the proton at C-9.



—Cont.

FIGURE 26.10 The biosynthetic conversion of squalene to cholesterol proceeds through lanosterol. Lanosterol is formed by a cyclization reaction of squalene-2,3-epoxide.

Step 4: A series of enzyme-catalyzed reactions converts lanosterol to cholesterol. The three highlighted methyl groups in the structural formula of lanosterol are lost via separate multistep operations, the C-8 and C-24 double bonds are reduced, and a new double bond is introduced at C-5.



Lanosterol Cholesterol

FIGURE 26.10 Cont.

PROBLEM 26.12 The biosynthesis of cholesterol as outlined in Figure 26.10 is admittedly quite complicated. It will aid your understanding of the process if you consider the following questions:

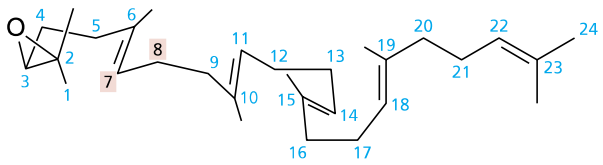
(a) Which carbon atoms of squalene 2,3-epoxide correspond to the doubly bonded carbons of cholesterol?

(b) Which two hydrogen atoms of squalene 2,3-epoxide are the ones that migrate in step 3?

(c) Which methyl group of squalene 2,3-epoxide becomes the methyl group at the C, D ring junction of cholesterol?

(d) What three methyl groups of squalene 2,3-epoxide are lost during the conversion of lanosterol to cholesterol?

SAMPLE SOLUTION (a) As the structural formula in step 4 of Figure 26.10 indicates, the double bond of cholesterol unites C-5 and C-6 (steroid numbering). The corresponding carbons in the cyclization reaction of step 2 in the figure may be identified as C-7 and C-8 of squalene 2,3-epoxide (systematic IUPAC numbering).



Coiled form of squalene 2,3-epoxide

PROBLEM 26.13 The biosynthetic pathway shown in Figure 26.10 was developed with the aid of isotopic labeling experiments. Which carbon atoms of cholesterol would you expect to be labeled when acetate enriched with ^{14}C in its methyl group ($^{14}CH_3COOH$) is used as the carbon source?

Once formed in the body, cholesterol can undergo a number of transformations. A very common one is acylation of its C-3 hydroxyl group by reaction with coenzyme A derivatives of fatty acids. Other processes convert cholesterol to the biologically important steroids described in the following sections.