1

Introduction

1.1   Steroids and Steroid Dimers

Steroids are a family of biologically active lipophilic molecules that include cholesterol, steroidal hormones, bile acids and plant sterols (also known as phytosterols). These metabolic derivatives of terpenes are biosynthesized by plants as well as animals including humans, and play an important role in biological systems (Li and Dias, 1997; Nahar *et al.*, 2007a). Structurally, a steroid is a lipid molecule having a carbon skeleton with four fused rings; three fused cyclohexane rings, known as phenanthrene, are fused with a cyclopentane ring (Sarker and Nahar, 2007). The basic tetracyclic seventeen carbon steroidal ring system is known as *1,2-cyclopentano-perhydrophenanthrene* or simply *cyclopentaphe-nanthrene* (*Figure 1.1.1*). All steroids are derived from the acetyl CoA biosynthetic pathway. The four rings are lettered A, B, C, and D, and the carbon atoms are numbered beginning in the A ring. In steroids, the B, C, and D rings always are *trans*-fused, and in most natural steroids, rings A and B also are *trans*-fused. Each member of the steroid family has a structure that differs from the basic cyclopentaphenanthrene skeleton in the degrees of unsaturation within the rings and the identities of the hydrocarbon side chain substituents, *e.g.*, alkyl, alcohol, aldehyde, ketone or carboxylic acid functional groups, attached to the rings.

Even minor changes in the functionalities attached to the steroid skeleton can lead to significant changes in their biological and pharmacological activities (Nahar *et al.*, 2007a). That is why synthetic chemists have always been keen to carry out structural modifications of steroids to optimize their biological and pharmacological properties or to discover new properties. Steroid dimers are one of such group of modified steroids that are well known for their rigid, predictable and inherently asymmetric architecture.

Steroid dimer formation was first noticed during photochemical studies on steroids. During the investigation of the effect of sensitized light on the activation of ergosterol (**1**) in the absence of oxygen, it was discovered that in an alcoholic solution containing sensitizer, ergosterol on exposure to sunlight had undergone dehydrogenation to form a strongly
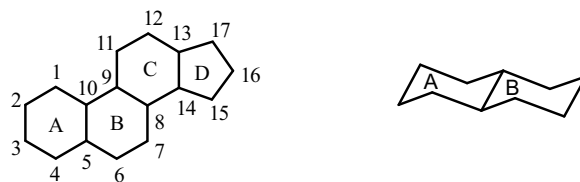
Figure 1.1.1 *Cyclopentaphenanthrene skeleton (left) and* trans-*fused rings (right)*

levorotatory substance ($[\alpha]_D$: $-209°$, mp: 205 °C) having double the original molecular weight and two hydroxyl groups. This bimolecular product was named bisergostatrienol (**2**) (*Scheme 1.1.1*) (Windaus and Borgeaud, 1928). Since this discovery, several dimeric steroids have been found in nature, particularly from marine sponges, and also have been synthesized in the laboratory (Nahar *et al.*, 2007a).

Steroid dimers can be classified broadly into acyclic dimers (also known as *'linear dimers'*) and cyclic dimers (*Figure 1.1.2*). Acyclic dimers involving connections between A, B, C or D rings, or *via* C-19, direct or through spacers, form the major group of steroid dimers (see *Chapter 2*). In the cyclic steroid dimers, dimerization of steroids, direct or through spacers, leads to formation of new ring systems or macrocyclic structures, *e.g.*, cyclocholates or cholaphanes, respectively (see *Chapter 3*). Steroid dimers can also be classified as symmetrical and unsymmetrical dimers; when a dimer is composed of two identical steroid monomeric units, it is called a symmetrical dimer, and when two different monomeric steroid units are involved or two identical monomeric steroid units are joined in a way that there is no symmetry in the resulting dimer, the dimer is known as an unsymmetrical dimer (*Figure 1.1.2*). One other way of classifying steroid dimers is to divide them into natural and synthetic dimers (*Figure 1.1.2*).

## 1.2 General Physical and Spectroscopic Properties of Steroid Dimers

In general, like most monomeric steroids, steroid dimers are lipophilic in nature and are not water soluble. However, depending on the monomeric steroid, spacer group or other
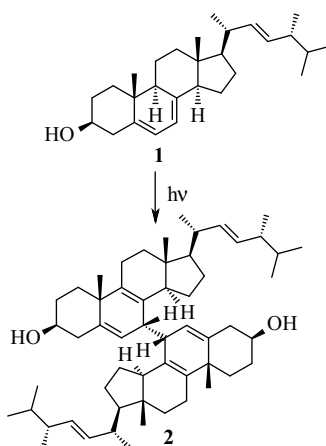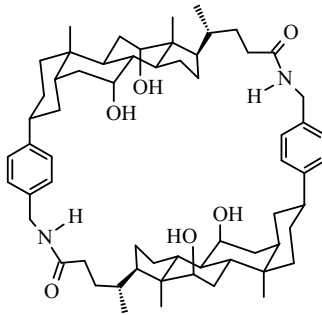

Scheme 1.1.1 *Conversion of ergosterol (**1**) to bisergostatrienol (**2**)*
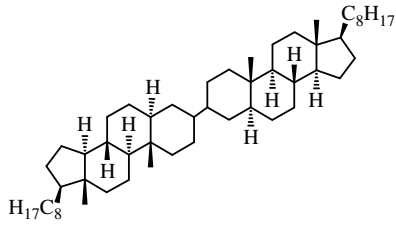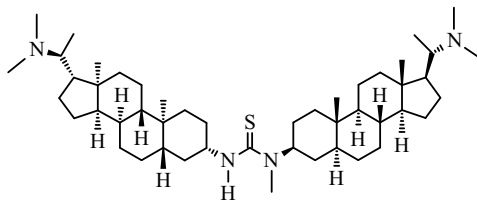
Bischolestane, a synthetic symmetrical acyclic (linear) steroid dimer
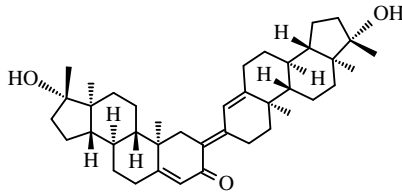
17α-methyltestosterone dimers, a synthetic acyclic unsymmetrical steroid dimer


Cholaphane, a synthetic cyclic (macrocyclic) steroid dimer

Japindine, a natural symmetrical steroid dimer

Figure 1.1.2 *Classification of steroid dimers*

functionalities present on the dimeric steroid skeleton, the solubility of such molecules can be quite variable. For example, steroid dimers composed of two sterol (steroid alcohol) units where the hydroxyl groups are not altered, as in bisergostatrienol (**2**), will retain some degree of polar character due to the hydroxyl groups, while keeping its nonpolar or hydrophobic nature because of the ring systems and other alkyl substituents or aliphatic side chains, and thus, these dimers will have properties like amphipathic lipids.

Most dimeric steroids are solids and can be transformed into well-formed crystals from various solvents (see *Chapters 2* and *3*), *e.g.*, bis[estra-1,3,5(10)-trien-17-on-3-yl]oxalate (**3**) was crystallized from $CHCl_3$-EtOAc (2:1) (Nahar, 2003).
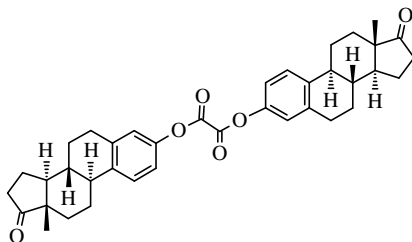

Bis[estra-1,3,5(10)-trien-17-on-3-yl]oxalate (**3**)

The melting points of steroid dimers are quite variable and depend on the monomer, the spacer groups and other functionalities. The UV absorption spectra of steroid dimers depend on the presence or absence of chromophores, *e.g.*, conjugated double bonds. The IR spectra can be different from dimer to dimer based on the functional groups present. Details on these spectral data of various steroid dimers will be presented in *Chapters 2–5*. Like the monomeric steroids, the dimeric steroids have several chiral centres in the molecule that make these molecules optically active. Therefore, specific rotation $[\alpha]_D$ data can provide additional characteristic information for any dimer.

To determine the molecular weight and molecular formula of steroid dimers, it is often essential to employ soft ionization techniques like fast-atom bombardment (FAB), electrospray ionization (ESI) or chemical ionization (CI) mass spectroscopy. The use of the MALDI–TOF technique has also been observed for some dimers very recently. MS information is particularly important for the symmetrical dimers composed of two identical steroid momoners without any spacer groups, where the information obtained from the nuclear magnetic resonance (NMR) spectroscopy may not be adequate to confirm the structure.

A range of 2D NMR techniques, particularly, correlation spectroscopy (COSY), nuclear Overhauser spectroscopy (NOESY), heteronuclear multiple bond coherence (HMBC) and heteronuclear single quantum coherence (HSQC), could be useful to confirm the structures of a number of dimeric steroids (Nahar, 2003; Nahar and Turner, 2003; Nahar *et al.*, 2006, 2007b). Sometimes, the use of the rotating frame Overhauser effect spectroscopy (ROESY) could be useful in establishing the relative stereochemistry, as in the case of crellastatins (D'Auria *et al.*, 1998; see *Chapter 4*). Fuzukawa *et al.* (1996) used ^{15}N-HMBC NMR technique to determine the orientation of the steroidal

units about the pyrazine ring in ritterazine A (**4**). However, the use of the ^{15}N-HMBC NMR technique is rather limited.
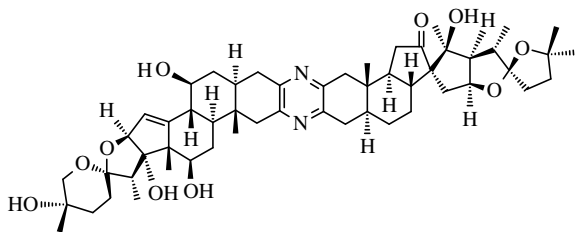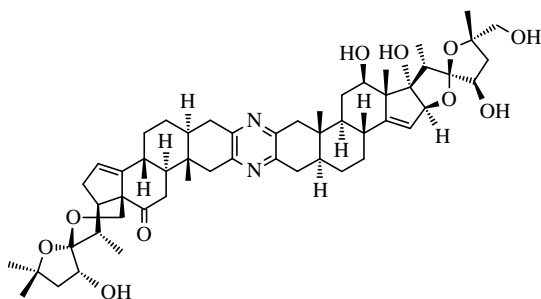

Ritterazine A (**4**)

1.3   Chromatographic Behaviour of Steroid Dimers

Most steroid dimers are nonpolar in nature and can be separated by normal-phase column, flash or thin layer chromatography (FCC or TLC) on silica gel (SiO$_2$) as the stationary phase and using various solvent mixtures, *e.g.*, *n*-hexane-EtOAc or CHCl$_3$-MeOH, as the mobile phase or eluent (Nahar, 2003). However, alumina or celite as the stationary phase has also been utilized for the separation of several steroid dimers.

On the TLC plates, steroid dimers can be detected by I$_2$ vapour, or using various sprays reagents, *e.g.*, vanillin-H$_2$SO$_4$ and Liebermann–Burchard reagents. For the detection of steroidal alkaloid dimers, *e.g.*, cephalostatin 1 (**5**), any alkaloid-detecting reagents, *e.g.*, Dragendorff's reagent, may be used.

The use of the reversed-phase high-performance liquid chromatography (HPLC) can equally be useful, and generally, MeOH-H$_2$O or MeCN-H$_2$O as the mobile phase, and a C$_{18}$ reversed-phase column as the stationary phase can be used (Nahar, 2003). However, for the purification of some cephalostatins and ritterazines, a C$_8$ reversed-phase column was reported to be used (see *Chapter 4*).

Cephalostatin 1 (**5**)

In some cases, for the initial separation of naturally occurring cytotoxic steroid dimers, *e.g.*, cephalostatins or ritterazines, solvent partitioning methods and droplet countercurrent chromatography (DCCC) have been regularly employed (see *Chapter 4*).

1.4   Applications of Steroid Dimers

Dimerization of steroid skeleton renders some unique characteristics that are applicable to different areas. Dimeric steroids have miceller, detergent, and liquid-crystal properties, and have been used as catalysts for different types of organic reactions. A number of dimeric steroids, *e.g.*, cephalostatins [*e.g.*, cephalostatin 1 (**5**)], are among the most potent natural cytotoxins. It has been suggested that a polyamine dimeric steroid binds to DNA due to the presence of two parts, one hydrophilic (positively charged nitrogen) and the other is hydrophobic steroid skeleton. Steroid dimers have also found their applications as *'molecular umbrella'* for drug delivery. Applications of steroid dimers are discussed further in *Chapter 6*.

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