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Cycloartenyl Palmitate: A Naturally Occurring Ester that Forms a Cholesteric Mesophase‡

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Abstract—Cycloartenyl palmitate has been isolated from banana peel (*Musa sapientum*) and from the seeds of *Strychnos nux-vomica*. This ester forms a cholesteric mesophase. The ester was carefully purified from each of these sources and identified by comparison of chemical, physical and mass spectral properties with an authentic sample of cycloartenyl palmitate. The isolation of this cholesteric ester is of interest since such substances have not been reported in plants and have been found only rarely in animal tissue.

Introduction

Recent interest in liquid crystals has prompted research on the properties of these intriguing substances. The cholesteric group of liquid crystals, most of which refract light when melted to form brilliant colors, are probably the least understood.[1] This is primarily due to the relatively few compounds exhibiting this behavior. Until recent reports of some 9,19-cyclopropane triterpene fatty acid esters that form the cholesteric mesophase,[2,3] various esters of cholesterol (I) and closely related forms[4] were the

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only steroid derivatives reported to behave in this manner.[5] These new cholesteric liquid crystals consist of the laurate, myristate and palmitate esters of 24ξ-methyl-9,19-cyclolanostan-3β-ol (24ξ-methyl cycloartanol) (II), and the palmitate ester of 4α,14α,24ξ-trimethyl-9,19-cyclocholestan-3β-ol (III). The corresponding esters of the respective C-24 unsaturated forms, 24-methylene cycloartanol (IV) and cycloeucalenol (V), are not cholesteric, while the palmitate ester of cycloartenol (9,19-cyclolanost-24-en-3β-ol) (VI) forms a cholesteric mesophase. Mesophase formation is thus dependent upon very critical steric requirements. These results have been discussed in detail elsewhere.[2,3]

Certain properties of liquid crystals would make them particularly well suited for living systems. These liquid crystals include both cholesteryl and other steryl esters (cholesteric) and various fatty acid forms (smectic). The possibility that liquid crystalline forms may play an important role in living systems has been discussed in detail, with particular emphasis on phospholipid interactions.[6] It is now well established that the general cellular membrane consists of a phospholipid bilayer that is modified by minor components, the most important being structural and functional (enzymic) protein.[7] The phospholipid component is thus of fundamental importance and the physico-chemical character of phospholipid systems is extremely significant to an understanding of both the structure and function of biological systems.

While phospholipid is found in nearly all living systems, few naturally occurring cholesteric liquid crystals are known. Their possible function in living systems is thus obscure. This paper reports the isolation of cycloartenyl palmitate from banana peel and the seeds of *Strychnos nux-vomica*. This triterpene ester forms a cholesteric mesophase.

Experimental

All reagents and solvents were analytical grade, the latter distilled before use. Melting points were determined on a Fisher-

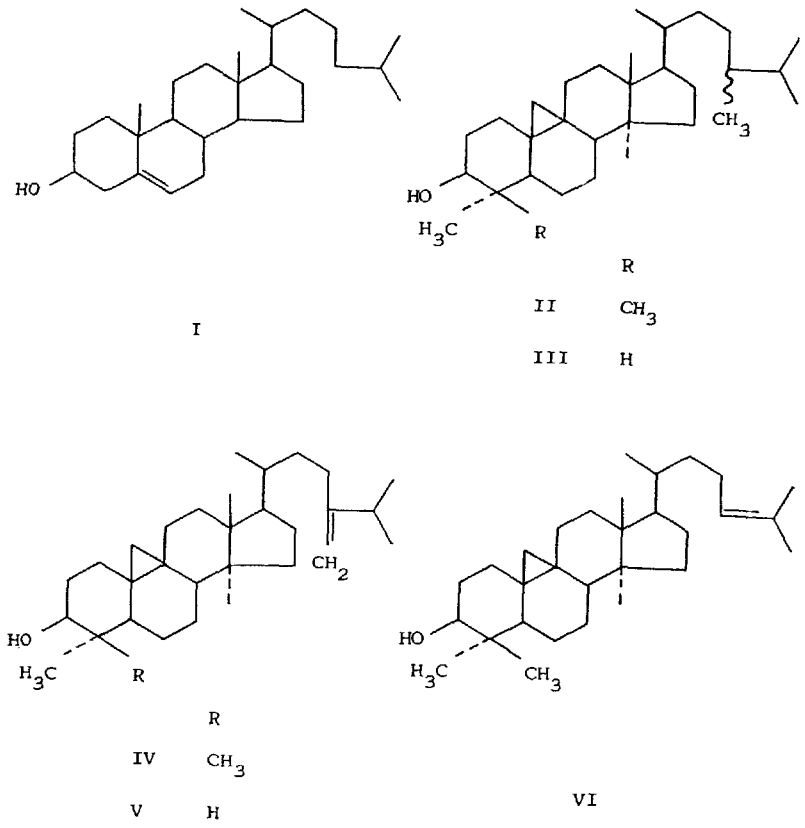

Figure 1. Structural formulas of (I) cholesterol, (II) 24ξ-methyl cycloartanol, (III) 4α, 14α, 24ξ-trimethyl-9,19-cyclocholestan-3β-ol, (IV) 24-methylene cycloartanol, (V) cycloeucalenol and (VI) cycloartenol. The configuration of the C-24 methyl group in II and III has not been determined.

Johns hot-stage apparatus and are uncorrected. Phase transition temperatures were obtained with a Nalge-Axelrod hot-stage polarizing microscope. The following abbreviations are used: FJ = Fisher-Johns, NA = Nalge Axelrod, iso = isotropic liquid, cho = cholesteric mesophase. Banana peels were obtained from bananas purchased locally and *Strychnos nux-vomica* seeds were obtained from S. B. Penick and Company, New York. Thin-layer chromatography (TLC) was performed using silica gel G spread 250 microns thick on glass plates in the usual manner.[8] Triterpene esters were chromatographed on silica gel containing silver nitrate (12%, w/w). The solvent systems used for development of the spotted plates were as follows: S-1, for triterpene esters, hexane-ether (93 : 7, v/v); S-2, for free triterpene alcohols, 2,2,4-trimethyl pentane-ethyl acetate-acetic acid (40 : 20 : 0.4, v/v/v). The compounds were detected on the plates by heating after being sprayed with anisaldehyde reagent. With S-1 homologous esters and saturated-unsaturated analogs were well separated (cycloartenyl acetate, R_f 0.27; cycloartenyl palmitate, R_f 0.64; cycloartanyl palmitate, R_f 0.90). Gas-liquid chromatographic analyses (GLC) were performed with a Barber-Colman Model 500 gas chromatograph equipped with a hydrogen flame detector. All phases and the support material (Gas Chrom Q, 100/120 mesh) were purchased from Applied Science Laboratories. For the separation of triterpene esters a 0.5 meter column of 3% SE-30 at 290° was used. The carrier gas was nitrogen with a flow rate of 100 cc/min. This system separates quite well homologous fatty acid esters.[9] The free triterpene alcohols were separated on a 2 meter column of 1% SE-30 at 238°, or a column of 3% XE-60 at 248° with argon as the carrier gas at a flow rate of 46 cc/min. Methyl esters of long chain fatty acids were separated on the SE-30 column at 130° or on a column of 3% SE-52 at 160°. Mass spectra were determined with an LKB Model 9000 single focusing instrument. The spectra were obtained by direct probe analysis under the following conditions: ion source, 260°; ionizing current, 60 μA; probe, 110°; ionizing energy, 70 eV.

Results

Dried seeds (10 kgm) of the tree *Strychnos nux-vomica* were extracted exhaustively with hot ethanol in a Soxhlet apparatus. Evaporation of the solvent yielded a viscous yellow oil which was dissolved in ether and washed with 5 per cent KOH to remove acidic material. The ether layer was washed several times with water, the ether evaporated and the neutral lipid (73 gm, 0.73% yield) then chromatographed on a column (5 × 180 cm) containing 1880 gm of Merck acid-washed alumina. The crude ester fraction was eluted from the column in the initial petroleum ether fractions. It consisted of 11.1 gm of viscous gum. Analysis by TLC on silver nitrate plates (S-1) indicated a major (95%) and a minor component (R_f 0.64 and 0.50, respectively). Separation of these two esters was achieved by chromatography on a column (2 × 60 cm) containing 130 gm of Celite-silica gel-silver nitrate (10 : 10 : 8, w/w/w). The column was eluted with petroleum ether followed by 2% benzene in petroleum ether. The major material was eluted in the latter fractions and crystallized from methanol-ether as fine micro needles, m.p. 52–54° (FJ). When the melt cooled, a deep violet color was observed, indicative of the formation of a cholesteric mesophase. The color formed by this ester is illustrated in Fig. 2. The material was observed between cover-slips with a Nalge-Axelrod hot-stage polarizing microscope. The crystalline solid melted directly to the isotropic liquid at 54–55°. Upon cooling the melt, a bright birefringence appeared at 53° (iso → cho). The color was dull blue-green with the polaroids crossed at 90° and a pink color with the polaroids aligned. Birefringence disappeared when the mesophase was heated to 52.5° (cho → iso). When the birefringent liquid was cooled further, a cover-slip displacement resulted in the formation of a violet colored plane texture. The color formation is thus monotropic, since the cholesteric phase is formed only upon cooling the isotropic liquid. Mesophase formation by this ester is extremely sensitive to solvent vapors. This is evidently similar to the effect of solvent vapor on the mesomorphic behavior of cholesteryl esters

(5,12). When a small amount of acetone is added to the isotropic melt and the sample is allowed to cool, the mesomorphic properties of the sample are altered dramatically. Upon cooling, a series of brilliant colors are observed (Fig. 2). The sample then cools to a blue color stable at room temperature. The color of such preparations has remained stable for several months. This effect has not been studied in detail, and no other solvents have been tested. An authentic sample of cycloartenyl palmitate was prepared by esterification of the triterpene with palmitoyl chloride as previously described[2,3] (cycloartenyl palmitate, m.p. 52–54° (FJ), 56–57° (NA) iso → cho 52°, blue mesophase). The ester gave a single spot on TLC (S-1, R_f 0.54) and co-chromatographed with cycloartenyl palmitate on GLC using a 1% SE-30 column. The ester had an absolute retention time (RT) of 7.5 min (cycloeucalenyl palmitate, RT 7.0 min; 24-methylene cycloartanyl palmitate, RT 8.0 min).

The mass spectrum of the ester is shown in Fig. 3, and is essentially identical to that shown for cycloartenyl palmitate. After the mass peak at m/e 664 and a peak at m/e 649 corresponding to M-CH_3, no fragment was observed until m/e 408, corresponding to loss of palmitic acid (256). Other fragments at m/e 393 (M-palmitic acid-CH_3), 365, 339, 297 (M-side chain-palmitic acid) and 286 are typical of the fragmentation pattern of cycloartenol.[10]

The ester was hydrolyzed in ethanol-benzene-water (80 : 10 : 10, v/v/v) containing 15% KOH. The mixture was refluxed one hour and the non-saponifiable fraction obtained in the usual manner. A single component was indicated by TLC in the region of the 4,4-dimethyl triterpenes (S-2, R_f 0.62). The triterpene crystallized as needles from acetone, m.p. 106–109° (Literature, cycloartenol m.p. 95°, solvated; 115°, anhydrous).[11] The triterpene also co-chromatographed with an authentic sample of cycloartenol (a gift of Dr. G. Ourisson, Strasbourg, France) on both 1% SE-30 and 3% XE-60 columns. The alkaline fraction remaining from the saponification mixture was acidified with hydrochloric acid and extracted thoroughly with ether. Following evaporation of the solvent, the residue was methylated with boron trifluoride-

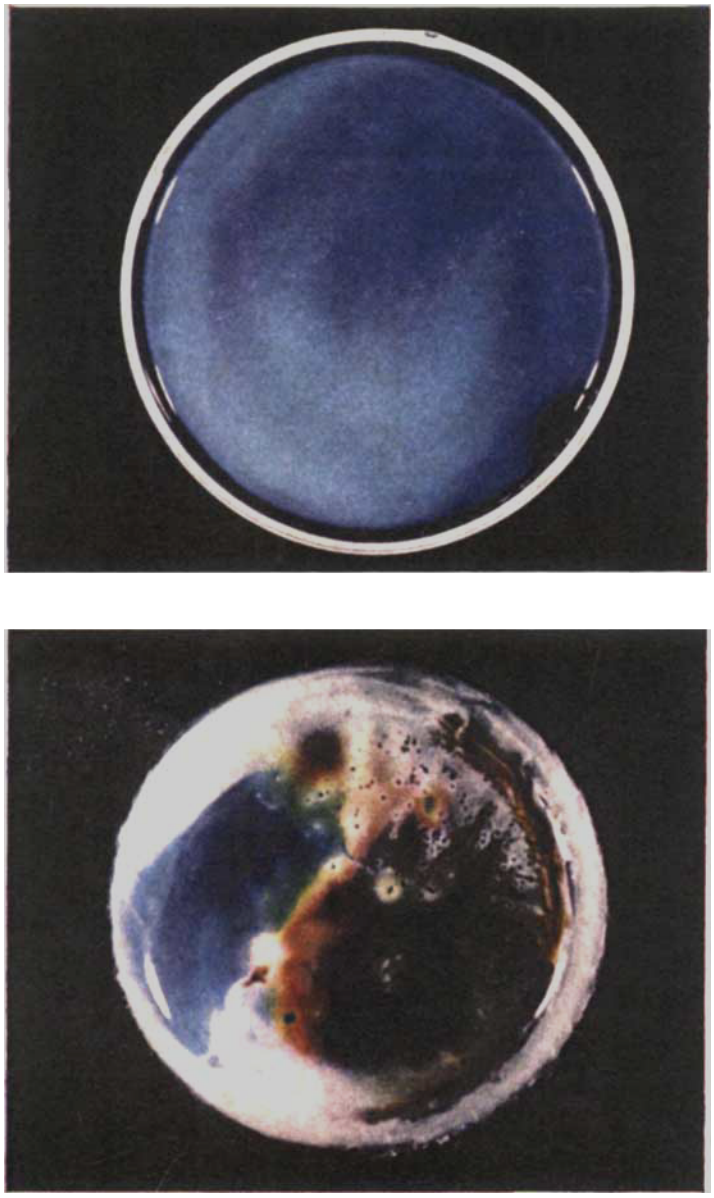

Figure 2. (A) Color associated with the mesophase of the cholesteric ester from *Strychnos nux-vomica* seeds. (B) A " lyotropic " type system formed with this ester containing a small amount of acetone. The samples were melted and allowed to cool to their isotropic → cholesteric transition temperatures at which time these colors were observed.

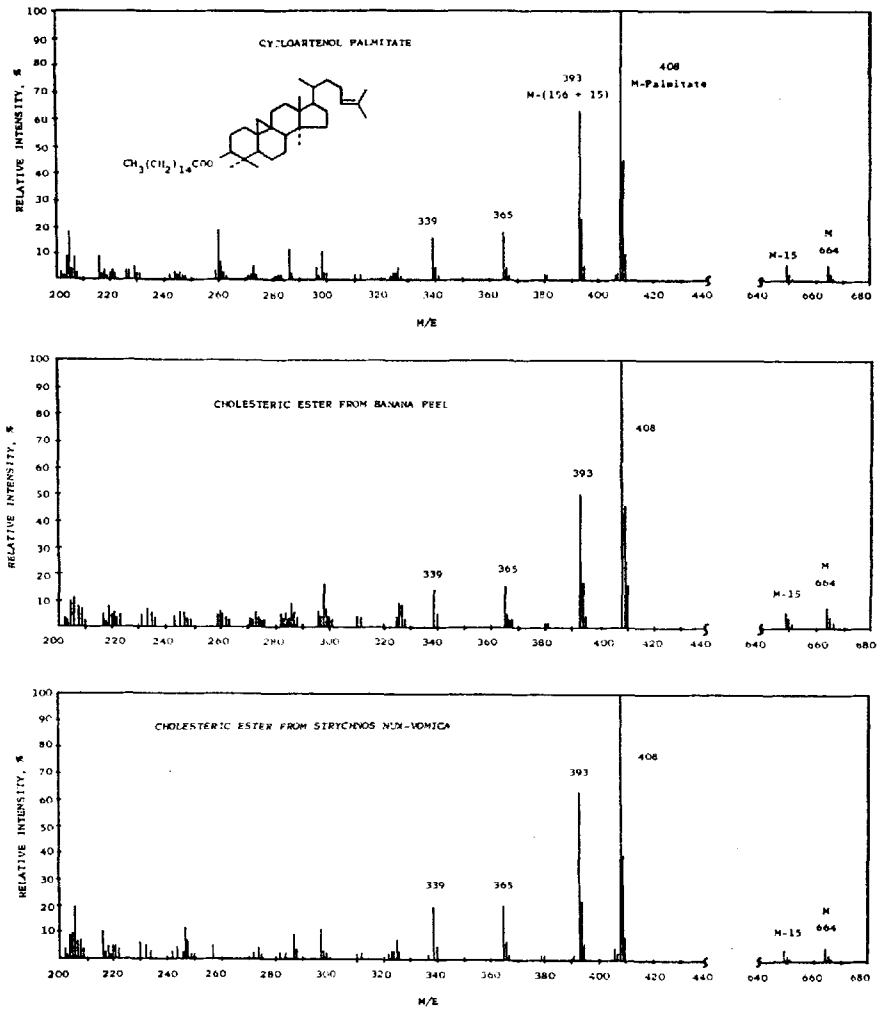

Figure 3. The 70 eV mass spectra of cycloartenyl palmitate and the cholesteric esters from banana peel and *Strychnos nux-vomica* seeds.

methanol. The methyl ester had the same retention time as methyl palmitate on 1% SE-30 and 3% SE-52 columns. The cholesteric ester present in *Strychnos nux-vomica* has thus been identified as cycloartenyl palmitate.

The ester fraction from the lipid of dried banana peels also contained a minor component which is cholesteric. Dried banana peels (14 kgm) were extracted exhaustively with ethanol and the neutral lipid obtained in the manner as described earlier. The lipid from banana peel consisted of 70 gm (5% yield) of an orange wax. This material was chromatographed on 1500 gm of alumina and the ester fraction eluted with petroleum ether. Material from the ester fraction was crystallized from acetone to yield a white amorphous solid which turned violet when melted. TLC indicated the presence of a major component (R_f 0.54) and several less polar minor components. Repeated crystallization of the mixture from either acetone or methanol-ether provided the major component, 24-methylene cycloartanyl palmitate.[2,13] The cholesteric substance was found in the filtrates in small amounts (R_f 0.64), and was eventually purified by chromatography on Celite-silica gel-silver nitrate as previously described. It represented from 10 to 12 per cent of the original ester mixture, and was identical in physical, chemical and optical properties to cycloartenyl palmitate. The mass spectrum of this ester is also shown in Fig. 2.

Discussion

The general model which has been proposed to explain the mechanical properties of liquid crystalline forms in biological systems consists of a solid core surrounded by a nematic layer which is then encompassed by a smectic layer.[6] Such a smectic layer consists of a complex of phospholipid molecules. The energetics of this model have been calculated and indicate that such a system is capable of responding with a great deal of sensitivity to various external stimuli. These include temperature, light, mechanical pressure, sound and the chemical environment. In addition, liquid crystals could also function in smell since the

properties of such systems are altered as a response to extremely small amounts of vapor.[12] This is an example of a response to the chemical environ of the liquid crystalline surrounding.

Tien has shown that a bimolecular lipid membrane may act as an energy transducer.[13] Such a membrane formed by chlorophyll exhibits a photovoltaic effect and photoconductivity when the membrane is exposed to strong white light. There is thus an increasing amount of evidence suggesting that liquid crystalline forms play an important role in biological systems.

While such smectic systems are relatively common, cholesteric liquid crystals have only rarely been found. Cholesteric forms have been identified in plasma and tissue of normal and atheromatous patients and are stable at body temperature.[14] A review of this work and the occurrence of liquid crystalline forms in the ovary, adrenal and other tissues has been published.[15] Cholesteryl esters have also been found in part of the eye (tapetum lucidum) of the oppossum (*Didelphis virginiana*),[16] where their possible function in light sensitivity should be considered.

The isolation of this cholesteric ester from two unrelated plant sources suggests that such substances are common in nature. Perhaps those plants which contain cycloartenol also contain long chain esters of this triterpene which are cholesteric. Since the initial procedure for the identification of plant sterols is generally a rather strong saponification step, these esters are obviously destroyed and have thus escaped detection in the past. Due to their unique optical properties, such substances may play an important biological function in the plants in which they are found.

In some plants, however, cycloartenol and related triterpenes are known to be esterified, although the esters themselves were not isolated and purified. From a crude ester fraction from birchwood Bergmann *et al.*[17] identified cycloartenol and other triterpenes. Cycloartenol has also been found in ester fractions of maize,[18] pea leaves,[19] grapefruit peel[20] and tobacco leaves.[19] In all of these instances only the crude ester fraction was obtained. This was hydrolyzed and the neutral components then identified. We are now re-investigating these tissues in an attempt to isolate

and purify these esters. Such studies are also being expanded to screen the ester fractions from a large number of plants for possible mesomorphic behavior. The cellular distribution and subcellular localization of cycloartenyl palmitate in banana peel and various parts of the *Strychnos nux-vomica* plant are being determined. These studies may give a clue to the possible biological function of these interesting substances.

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