Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl16

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Version of record first published: 21 Mar 2007.

To cite this article: Ahmed M. Atallah & Harold J. Nicholas (1973): Liquid Crystalline Properties of Some Pollinastanol Fatty Acid Esters, Molecular Crystals and Liquid Crystals, 24:3-4, 213-222

To link to this article: http://dx.doi.org/10.1080/15421407308084232

Molecular Crystals and Liquid Crystals. 1973. Vol. 24, pp. 213–222

Printed in Great Britain

Liquid Crystalline Properties of Some Pollinastanol Fatty Acid Esters†

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Received September 28, 1972; *in revised form January* 30, 1973

Abstract—Pollinastanol, the only known naturally occurring 3-desmethyl sterol possessing a 9,19-cyclopropane ring, is capable of exhibiting liquid crystalline properties, cholesteric and smectic, when esterified with certain even-chain acids. The decanoate and the palmitate exhibited a markedly colored cholesteric mesophase, while the laurate and myristate showed no colors except in the presence of traces of solvent. These specific mesophase formations are believed to be due to the distinctive structure of pollinastanol, which is intermediate between that of the tetracyclic triterpene 31-norcycloartanol[2] and cholesterol[3,4] both known to form distinct liquid crystalline mesophases. Pollinastanol was isolated from the pollen of *Taraxacum dens leonis* where it is present in minute quantities mainly in esterified form. Its possible presence as a naturally occurring liquid crystal is discussed.

1. Introduction

Recent publications from this laboratory have established the mesomorphic properties of a number of new triterpene esters, largely even-chain fatty acid esters of tetracyclic triterpenes.[1,2,5–8] Certain structural characteristics of the side chain have been found to be critical with regard to mesophase formation. We now wish to report some observations on a series of fatty acid esters of pollinastanol (I), a sterol which is identical in structure to cholesterol (V) except for the absence of unsaturation, the presence of a 9,19-cyclopropane ring and a methyl group at C-14.

2. Results

All synthesized even-chain fatty acid esters of pollinastanol (acetate,

† Paper III of a series "Naturally occurring liquid crystals", Paper I and II of this series are Refs. (1) and (2).

hexanoate, decanoate, laurate, myristate and palmitate), were capable of mesophase formation.

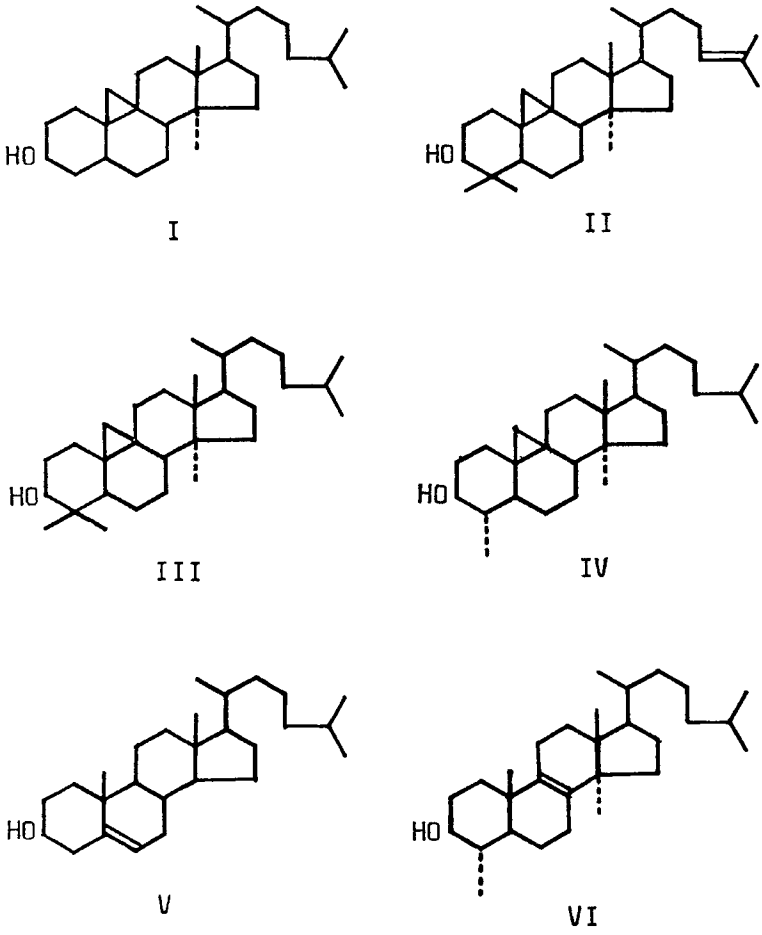
The acetate and hexanoate formed monotropic colorless-cholesteric mesophases whereas the decanoate, laurate, myristate and palmitate showed both smectic and cholesteric transitions. The decanoate was the only ester exhibiting enantiotropic mesophase transitions. The colors associated with the cholesteric phase produced by heating dry crystals of the decanoate and palmitate derivatives were produced only upon shearing whereas the laurate and myristate behaved like the acetate and hexanoate in producing no colors even upon application of mechanical stress to their cholesteric mesophases. When the decanoate, laurate, myristate and palmitate were eluted from the chromatography column with benzene, during the purification process, and the solvent carefully removed by evaporation, the noncrystalline residue obtained upon cooling at room temperature exhibited a beautiful greyish-blue color. The palmitate showed the usual rainbow of colors lasting several hours. This appears to be a combined thermotropic-lyotropic effect. The acetate and hexanoate did not show this phenomenon and are thus classified as colorless cholesterics.

Microscope Examination of Pollinastanol Esters Phase transitions were observed under crossed polarizers and color formation observed with the naked eye.

Pollinastanyl Acetate m.p. 85 °C. The melt passed directly to the isotropic phase. On cooling to 48 °C a strongly birefringent cholesteric mesophase (focal-conic) appeared which lasted only till 47 °C followed by crystallization at 46 °C. Cover slip displacement of the cholesteric phase caused no colors to appear.

Pollinastanyl Hexanoate m.p. 75–77 °C. The melt passed to the isotropic liquid. On cooling to 58 °C a strongly birefringent colorless cholesteric mesophase (focal-conic) appeared. Mechanical disturbance did not bring about any colors. The preparation crystallized at 55 °C.

Pollinastanyl Decanoate m.p.44° C. Crystals melted to a birefringent flowing melt which became isotropic at 49 °C. Cooling the preparation to 47 °C resulted in the plane cholesteric mesophase associated upon cover slip displacement with a play of colors ranging from a red, yellow, to green and finally to blue as seen by the naked



FORMULAE I–VI

eye. In additional tests in which the preparations were left to cool undisturbed, the cholesteric plane texture was seen to change to a characteristic smectic structure at 45 °C followed by crystallization at 36 °C.

Pollinastanyl Laurate m.p. 51 °C. The melt passed directly to the isotropic liquid. On cooling to 47 °C the birefringent cholesteric phase was formed but cover slip displacement caused no color appearance. The smectic phase started to be observed at 46 °C with typical large focal-conics and crosses scattered in the field. Crystallization occurred from the mesophase at 32 °C.

Pollinastanyl Myristate m.p. 58–59 °C. The isotropic liquid was formed directly from the melt. Cooling at 48 °C caused the birefringent focal-conic cholesteric mesophase to appear. This phase changed at 46 °C to a much brighter focal-conic smectic phase similar to that given by the laurate. No colors were observed from the preparation even upon mechanical disturbance. Crystallization occurred at 30 °C.

Pollinastanyl Palmitate m.p. 59–60 °C. The crystals melted directly to the isotropic liquid. A strongly birefringent cholesteric mesophase was formed at 49 °C. This was followed by the smectic mesophase at 48 °C. Upon shearing of the cholesteric mesophase a beautiful rainbow, of red, green, yellow and blue colors was observed. Crystallization of spherulites occurred at 47 °C. In another experiment the birefringent mesophase was not disturbed. In this case no colors were seen, perhaps because the transition to the smectic mesophase was very rapid and again crystallization occurred at 47 °C. The existence of both the cholesteric (negative optical sign) and smectic (positive optical sign) mesophases for pollinastanyl palmitate was ascertained by conoscopic observation of the optical sign of birefringence[9] (see experimental section).

Phase transitions of pollinastanol esters are diagramatically expressed in Fig. 1.

3. Experimental Section

General

Melting points were determined on a Fisher–Jones hot stage apparatus and are corrected. Phase-transition temperatures were determined

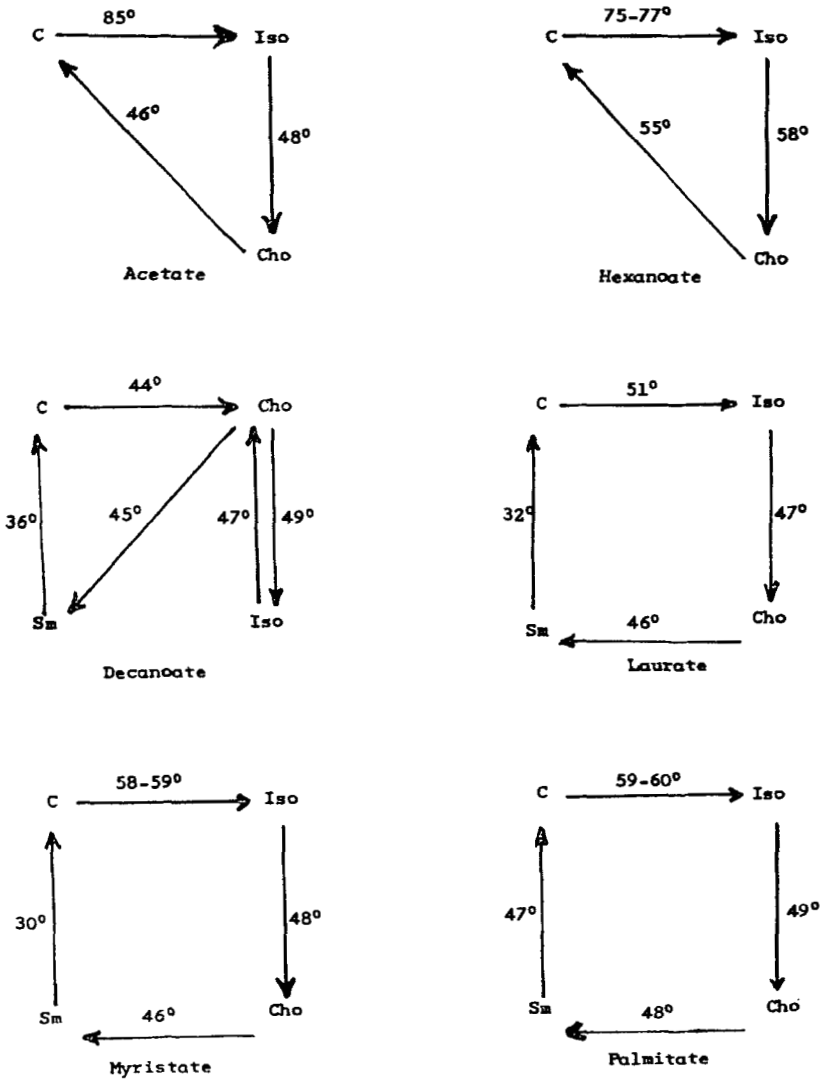


Figure 1. Phase transition temperatures of pollinastanol esters.

on a Nalge–Axelrod hot-stage polarizing microscope equipped with a 75 watt white light bulb. Samples for inspection were placed on a thoroughly cleaned glass cover slip. After fusion, the melt was covered with an additional cover slip so that the melt was uniformly

distributed between cover slips. Air bubbles and dust were avoided during microscopic examination.

Determination of type of mesophase by conoscopic observation for pollinastanyl palmitate was kindly performed in Dr. Wolfgang Elser's laboratory at the United States Army Electronic Command, Night Vision Laboratory, Fort Belvoir, Virginia, on a Mettler FP-2 hot stage microscope, by Mr. Arlin J. Brown.

All chemicals were analytical grade. Solvents were distilled before use, except ethanol used for extraction of the pollen. The petroleum ether used had a boiling point of 30–60 °C. Pollen of *Taraxacum dens leonis* was supplied by Mr. Louis de Lescure, Apiculteur, Laverne, Severac-le-Chateau, France. A sample of authentic pollinastanol was kindly supplied by Prof. Michel Barbier.

Chromatography and Mass Spectrometry All chromatographic and mass spectrometric procedures and materials used (column chromatography (CC), thin-layer chromatography (TLC), gas-liquid chromatography (GLC) and gas-liquid chromatography–mass spectrometry (GLC–MS)) were the same as described for the isolation, purification, purity determination and identification of 31-norcycloartanol[2] and its fatty acid esters.

Phase Transitions

Microscopical changes were observed under crossed polaroids. Colors were observed by the naked eye at glancing angles on the microscopic stage. Phase transitions are abbreviated as follows: isotropic: iso, smectic: sm, cholesteric: cho. Arrows show the direction of change, e.g.: iso $\rightarrow$sm, etc.

Isolation and Purification of Pollinastanol The neutral lipid fraction of pollen of *Taraxacum dens leonis* was obtained as previously described[2] for the isolation of 31-norcycloartanol. 200 g of this fraction was chromatographed on a 7 cm diameter column containing 2000 g neutral aluminum oxide. Hydrocarbons were eluted by washing the column with 2000 ml petroleum ether. This was followed by 3000 ml of benzene which eluted the ester fraction. Further fractionation of this column with increasing proportions of ethyl ether in benzene mixtures afforded 4,4-dimethyl, 4α-methyl and 4-desmethyl sterols. Concentration of the 4-desmethyl sterols by repeated column chromatography and preparative TLC was performed.

Attempts to isolate pollinastanol from this fraction by the method reported by Devys and Barbier[10] showed that this sterol was present only in very minimal quantities in the free form. Therefore the ester fraction eluted from the column was saponified with 15% alcholic KOH. The nonsaponifiable fraction (23 g) was chromatographed on a 5 cm diameter containing 500 g neutral aluminum oxide. Fractions containing 4,4-dimethyl and 4α-methyl sterols were eluted with petroleum ether–ethyl ether 6 : 4. The 4-desmethyl sterols were eluted next with the same mixture of solvents in the ratio 4 : 6.

Separation of Δ^5 from Δ^7 sterols (pollinastanol co-chromatographed with Δ^5 sterols) was effected by preparative TLC on silica gel G plates using pentane-ethyl acetate 7 : 3.[10] The Δ^5 sterol bands were extracted with ethyl ether and the residue of the ethereal solution crystallized from methanol. The product thus obtained was found by GLC to consist of at least 90% pollinastanol. Repeated recrystallization from ethyl acetate gave 250 mg of pollinastanol of high purity (96%) judged by gas-liquid chromatography (GLC) and gas-liquid chromatography–mass spectroscopy (GLC–MS) and was identical to an authentic sample of pollinastanol kindly supplied by Prof. M. Barbier. The paucity of the material did not allow further purification at this point. Further purification of the compound was carried out on the synthesized pollinastanol esters as described below.

Pollinastanol Fatty Acid Esters 40 mg of pollinastanol were used to prepare each of the acetate, hexanoate, decanoate, laurate, myristate and palmitate as previously described for the preparation of 31-norcycloartanol esters.[2] Further separation of the individual esters from minor traces of a contaminent was performed on a 1 cm diameter glass column on 10 g of a mixture of silica gel G, celite, $AgNO_3$ 10 : 10 : 4. The esters were crystallized from acetone and thoroughly dried in an Abderhalden drying apparatus under 15 cm Hg vacuum pressure at the boiling point of acetone for 2 hours. The products so obtained were of a purity of 97% as tested by GLC.

4. Discussion

Pollinastanol I is one of the rarer plant sterols, having been detected in pollen[10] and recently in other plant material.[11] It

has been reported in the pollen of *Taraxacum dens leonis*[10,12] along with cycloartenol (II), cycloartanol (III), 31-norcycloartanol (IV), cholesterol (V), and 31-nordihydrolanosterol (VI).† Pollinastanol, the subject of this study, and certain esters of compounds (II), (III), (IV) and (V) are known to form liquid crystalline mesophases. This accumulation in one tissue of five sterols of different composition, yet closely related structurally to each other while all capable of forming liquid crystals, could be more than coincidental. The potential biochemical or physiological significance of this co-occurrence is under study in our laboratory.

Based on structural requirements known to be requisite (in the sterol nucleus) for cholesteric mesophase formation, and based on our own observations on steroidal side-chain requisites,[7] even-chain fatty acid esters of pollinastanol should be capable of exhibiting colored cholesteric mesophases. The side-chain of pollinastanol (I), for example, is identical in structure and configuration with that of cholesterol, cycloartanol and 31-norcycloartanol, all three of which form both smectic and cholesteric properties. Consequently we expected pollinastanol to exhibit such mesophases with even-chain fatty acids from C_2 to C_{16}. However, only the decanoate and palmitate of pollinastanol gave colored thermotropic transitions and this only upon cover slip displacement. At this time, however, it seems logical to describe the birefringent mesophases that appeared for the lower homologues of pollinastanol fatty acid ester as being cholesteric mesophases, although no colors were observed even upon shearing. This assignment seems justified since, as a rule, the short chain length homologues of cholesterol and other related compounds are cholesteric while the longer esters exhibit both smectic and cholesteric mesophases. Besides, no typical smectic structures were observed in the lower homologues.

In comparing the structural characteristics of cycloartanol (III), 31-norcycloartanol (IV) and cholesterol (V) and considering the influence of substituents on mesophase formation, it seems that the

† For results of tests on mesomorphic behavior of 31-nordihydrolanosterol which we identified in the pollen of *Taraxacum dens leonis* see A. M. Atallah and H. J. Nicholas: Influence of the position of ring unsaturation in Steroids and Triterpenes on type and formation of mesophase: Influence of Δ^8 double bond. *Mol. Cryst. and Liquid Cryst.* **18**, 339 (1972).

presence or absence of the nuclear double bond at C-5, the presence or absence of methyl substituents at C-4 and/or C-14 does not affect the potentiality of the cholesteric mesophase. Also the presence or absence of a 9,19-cyclopropane ring does not seem to be of special influence in this respect. Why not all of the even-chain fatty acid esters of pollinastanol prepared for this investigation are able to exhibit colored cholesteric mesophases is not clear. The most logical explanation at the present time may lie in the length of the fatty acid moiety of the esters, which seems to be of critical value for color formation. The difference in behavior of cholesteryl palmitate, which forms a colored cholesteric mesophase, and cholesteryl stearate, which does not, provides another representative analogy.[3]

The impurities present to the extent of 3% in our esters of pollinastanol, as judged by GLC, may have some influence on the transition temperatures but to a lesser extent on the type of mesophase. Furthermore since all the esters were of almost the same degree of purity the discrepancies in color formation along the series must therefore be due solely to a structural nature.

Pollinastanol was originally isolated from the nonsaponifiable portion of *Taraxacum dens leonis* pollen extracts.[10] However, we have found that the bulk of this sterol (95%) is present in the ester fraction. Examination of the fatty acid moieties of the ester fraction revealed the presence of lauric, myristic, palmitic and stearic acids.[2] Therefore pollinastanol is most likely present in this particular pollen as a naturally occurring liquid crystal, since it may be esterified with either lauric, myristic or palmitic acid, or with all three acids. The same applies to the presence of 31-norcycloartanol, cycloartenol and cycloartanol largely in the esterified form.[2] Therefore the natural presence of those compounds in a chemical combination with specific fatty acids may strengthen the view that liquid crystalline compounds have a physiological role in plants.

Acknowledgements

This research was supported by Grant No. GB-19113 from the National Science Foundation. We wish to thank Professor M. Barbier for a sample of authentic pollinastanol. We are indebted to Dr. Wolfgang Elser and Mr. Arlin J. Brown for the conoscopic examination of a sample of pollinastanyl palmitate.

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