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Chemistry

Rotational isomerism about the 17(20)-bond of steroids and euphoids as shown by the crystal structures of euphol and tirucallol

(conformational control/(20*R*)- and (20*S*)-sterols/cholesterol/20-isocholesterol/20-epicholesterol)

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ABSTRACT **The influence of configuration at C-20 on rotation about the 17(20)-bond in steroids and euphoids was examined by x-ray crystallographic studies of the C-20 epimers euphol and tirucallol. The H atom on C-20 was in back next to C-18 in the crystal structures of both of the compounds, and C-22 was found to be *cis*-oriented ("left-handed") to C-13 in euphol and *trans*-oriented to it ("right-handed") in tirucallol. The results, which are consistent with the known left-handed crystal structure of 24(25)-dihydroeuphol and right-handed crystal structure of cholesterol and other natural sterols, lend further credence to the earlier suggestion that rotational isomerism at the 17(20)-bond can arise in C-20 epimers and that there is preference for an arrangement with the 20-H atom adjacent to C-18.**

It is known from x-ray data (1–3) that in the crystalline state, sterols with the natural configuration (20α-H atom, usually 20*R* in the sequence rule) have a conformation about the 17(20)-bond such that, in the usual view of the molecule, C-22 is to the right. As we suggested earlier (4, 5), the preference for this "right-handed" rotational isomer probably derives from its having the smallest of the groups on C-20 (the H atom) in front and, therefore, adjacent to C-18 in a pseudo-1,3-diaxial fashion. If this were the correct explanation, the H atom on C-20 should remain adjacent to C-18 after inversion. This would place C-22 on the left ("left-handed" isomer).

We undertook a study of this stereochemistry, because the ability of the sterol side chain to assume a right-handed conformation without having a methyl group in front seems to be of biological importance. There is a clear correlation between the biological activities of (20*R*)-sterols (which can have this stereochemistry) and (*E*)-17(20)-dehydrocholesterol with C-22 fixed to the right (5–11). Similarly, the activities of (20*S*)-sterols correlate with (*Z*)-17(20)-dehydrocholesterol (5–10). If only one of the 17(20)-dehydrosterols is active, it is the right-handed (*E*)-isomer, and then of the C-20 epimers only the one with the (20*R*)-configuration is active (5–10). These correlations have been found in the ability of sterols to perform what is thought to be the bulk membrane function in yeast (5, 6), to be metabolized by a protozoan (7, 8), and to induce formation of oospores in an oomycete (9, 10). If, on the other hand, both of the 17(20)-dehydrosterols are active, as they are in depressing hepatic cholesterol synthesis after being fed to mice (11), then both of the C-20 epimers of cholesterol are active (11).

To determine whether inversion of the C-20 configuration of sterols is actually accompanied in the solid state by transfer of C-22 from the right to the left side of the molecule, we have tried to grow large enough crystals of 20-epicholesterol, prepared as described (12), to permit x-ray analysis. Unfortunately, crystals of adequate size have not been obtained, so we turned our attention to the naturally occurring (13) C-20 epimers euphol and tirucallol (Fig. 1). These tetracyclic triterpenoids, which did yield crystals of sufficient thickness, are in the euphoid series (14) having C-13, C-14, and C-17 inverted compared to the steroids (13, 14). If maintenance of the 20-H atom adjacent to C-18 were important, then the (20*S*)-isomer (tirucallol) should have a right-handed conformation (C-22 *trans* to C-13) as found in (20*R*)-sterols (e.g., lanosterol, cholesterol, etc.). Conversely, the (20*R*)-euphoid (euphol) should have a left-handed conformation as we presume (4, 5) is preferred by (20*S*)-sterols (e.g., 20-epicholesterol, etc.). In the crystal structure of 24(25)-dihydroeuphol, C-22 has indeed been found (15, 16) to be on the left (*cis* to C-13). We now report that in tirucallol C-22 is on the right and we confirm with euphol itself that, when the configuration is 20*R*, C-22 is on the left.

MATERIALS AND METHODS

Materials. *Euphorbia tirucalli* grown in California and kindly provided to us as a dried sample through the courtesy of M. Calvin (University of California, Berkeley) and Thomas Mock (South Coast Field Station, University of California, Santa Ana) was the source (17–20) of both euphol and tirucallol. A second sample of euphol was available from *Euphorbia cyparissias* (21) through the generosity of A. N. Starratt (Canada Department of Agriculture, London, ON).

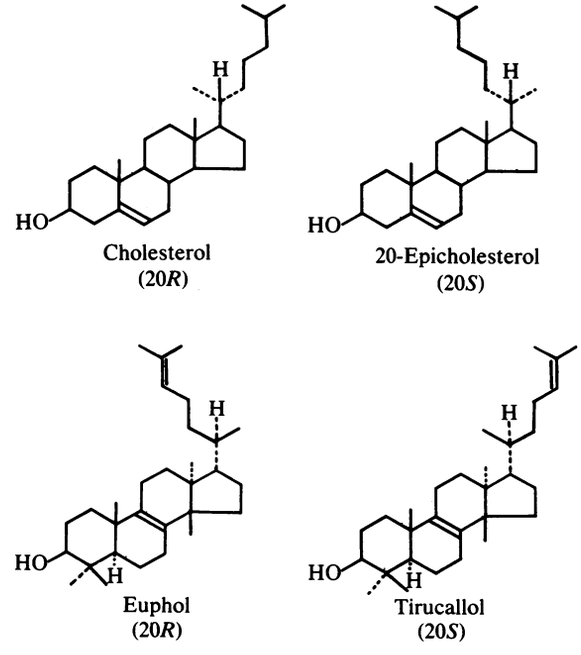

FIG. 1. Structures of C-20 epimeric pairs of steroids and euphoids.

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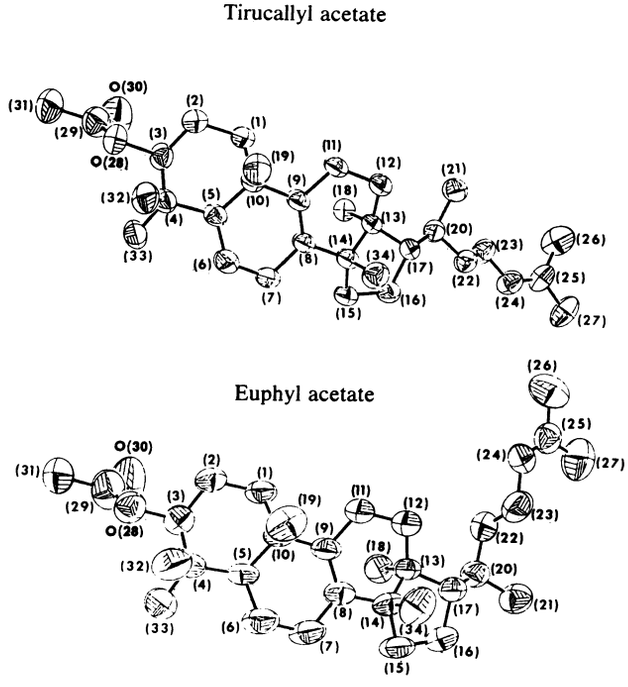

FIG. 2. Crystal structures of tirucallyl and euphyl acetates showing thermal ellipsoids for the carbon atoms, which were determined at the 50% probability level.

Table 2. Bond lengths (Å) for euphyl (I) and tirucallyl (II) acetates

	I	II		I	II
C-1/C-2	1.510 (7)	1.523 (5)	C-1/C-10	1.540 (6)	1.554 (5)
C-2/C-3	1.514 (7)	1.501 (5)	C-3/C-4	1.524 (6)	1.521 (5)
C-3/O-28	1.456 (6)	1.463 (4)	C-4/C-5	1.554 (7)	1.556 (4)
C-4/C-32	1.545 (7)	1.532 (5)	C-4/C-33	1.540 (7)	1.547 (5)
C-5/C-6	1.533 (6)	1.524 (5)	C-5/C-10	1.551 (6)	1.547 (5)
C-6/C-7	1.527 (7)	1.523 (4)	C-7/C-8	1.469 (6)	1.512 (5)
C-8/C-9	1.369 (6)	1.330 (4)	C-8/C-14	1.517 (7)	1.513 (4)
C-9/C-10	1.511 (6)	1.551 (4)	C-9/C-11	1.521 (7)	1.526 (5)
C-10/C-19	1.531 (6)	1.547 (5)	C-11/C-12	1.508 (7)	1.550 (4)
C-12/C-13	1.526 (6)	1.521 (5)	C-13/C-14	1.562 (6)	1.551 (5)
C-13/C-17	1.539 (6)	1.558 (4)	C-13/C-18	1.522 (6)	1.535 (5)
C-14/C-15	1.527 (7)	1.538 (5)	C-14/C-34	1.557 (7)	1.549 (5)
C-15/C-16	1.532 (7)	1.532 (5)	C-16/C-17	1.544 (6)	1.554 (5)
C-17/C-20	1.561 (6)	1.549 (5)	C-20/C-21	1.510 (7)	1.520 (5)
C-20/C-22	1.541 (7)	1.530 (4)	C-22/C-23	1.470 (8)	1.509 (5)
C-23/C-24	1.504 (8)	1.508 (5)	C-24/C-25	1.311 (7)	1.311 (5)
C-25/C-26	1.502 (8)	1.476 (7)	C-25/C-27	1.506 (8)	1.498 (5)
C-28/C-29	1.312 (7)	1.319 (5)	C-29/O-30	1.203 (8)	1.187 (5)
C-29/C-31	1.496 (8)	1.497 (6)			

Numbers in parentheses are estimated SD.

The physical properties of the two euphol samples were identical. Tirucallol was submitted to argentation chromatography for final purification as the acetate, which was then used for x-ray analysis. To maintain consistency, euphol was

Table 1. Atomic coordinates ($\times 10^4$) for euphyl and tirucallyl acetates

	Euphyl acetate			Tirucallyl acetate		
Atom	*x*	*y*	*z*	*x*	*y*	*z*
C-1	−387 (5)	10,922 (2)	9,277 (2)	5,422 (4)	1,776	3,242 (1)
C-2	−419 (5)	10,079 (3)	9,510 (2)	6,234 (3)	1,875 (4)	3,886 (1)
C-3	634 (5)	9,517 (3)	9,132 (2)	6,613 (3)	3,770 (4)	4,072 (1)
C-4	2,637 (5)	9,746 (3)	9,066 (2)	5,222 (3)	5,038 (3)	4,010 (1)
C-5	2,679 (4)	10,642 (3)	8,883 (2)	4,351 (3)	4,841 (3)	3,360 (1)
C-6	4,607 (6)	10,970 (3)	8,781 (2)	2,947 (3)	6,076 (4)	3,196 (1)
C-7	4,495 (6)	11,748 (3)	8,445 (2)	2,649 (3)	6,308 (3)	2,523 (1)
C-8	2,994 (4)	12,295 (3)	8,591 (2)	2,748 (3)	4,567 (3)	2,194 (1)
C-9	1,559 (5)	12,056 (2)	8,917 (2)	3,281 (3)	3,051 (3)	2,457 (1)
C-10	1,560 (5)	11,263 (2)	9,223 (1)	3,909 (3)	2,930 (3)	3,133 (1)
C-11	29 (6)	12,643 (3)	9,051 (2)	3,300 (3)	1,310 (3)	2,109 (1)
C-12	95 (6)	13,480 (3)	8,810 (2)	2,636 (3)	1,384 (3)	1,436 (1)
C-13	1,185 (5)	13,522 (2)	8,268 (1)	2,917 (3)	3,217 (3)	1,183 (1)
C-14	3,110 (5)	13,159 (3)	8,393 (2)	2,109 (3)	4,614 (3)	1,535 (1)
C-15	4,095 (6)	13,335 (3)	7,845 (2)	2,347 (3)	6,340 (3)	1,194 (1)
C-16	3,390 (5)	14,164 (3)	7,669 (2)	2,162 (3)	5,757 (4)	542 (1)
C-17	1,710 (5)	14,352 (3)	8,035 (2)	2,188 (3)	3,679 (4)	530 (1)
C-18	216 (5)	13,039 (3)	7,818 (2)	4,691 (3)	3,535 (4)	1,258 (1)
C-19	2,341 (7)	11,416 (3)	9,806 (2)	2,645 (3)	1,989 (4)	3,432 (1)
C-20	270 (6)	14,847 (3)	7,700 (2)	2,935 (3)	2,911 (4)	9 (1)
C-21	1,104 (7)	15,619 (3)	7,488 (2)	2,921 (4)	879 (4)	6 (1)
C-22	−1,553 (7)	15,023 (3)	7,997 (2)	2,094 (3)	3,652 (4)	−583 (1)
C-23	−1,458 (7)	15,552 (3)	8,487 (2)	2,894 (3)	3,323 (5)	−1,115 (1)
C-24	−3,240 (7)	15,638 (3)	8,794 (2)	2,065 (3)	4,199 (4)	−1,674 (1)
C-25	−4,507 (7)	16,186 (3)	8,727 (2)	1,888 (3)	3,596 (5)	−2,222 (1)
C-26	−6,210 (8)	16,201 (4)	9,076 (2)	2,476 (5)	1,846 (7)	−2,390 (1)
C-27	−4,410 (10)	16,853 (3)	8,304 (3)	1,003 (4)	4,640 (6)	−2,728 (1)
C-28	544 (4)	8,709 (2)	9,363 (1)	7,391 (2)	3,795 (4)	4,691 (1)
C-29	−763 (7)	8,235 (3)	9,184 (3)	8,934 (4)	3,786 (5)	4,813 (2)
C-30	−1,884 (6)	8,444 (2)	8,847 (2)	9,746 (3)	3,739 (7)	4,440 (1)
C-31	−742 (7)	7,441 (3)	9,478 (3)	9,741 (4)	3,776 (6)	5,472 (2)
C-32	3,719 (6)	9,572 (3)	9,606 (2)	5,840 (4)	6,955 (4)	4,101 (1)
C-33	3,462 (7)	9,216 (3)	8,603 (2)	4,183 (3)	4,677 (5)	4,491 (1)
C-34	4,137 (7)	13,607 (3)	8,870 (2)	319 (3)	4,320 (4)	1,479 (1)

Numbers in parentheses are estimated SD.

Table 3. Bond angles (degrees) for euphyl (I) and tirucallyl (II) acetates

	I	II		I	II
C-2/C-1/C-10	112.8 (3)	111.9 (2)	C-1/C-2/C-3	110.2 (4)	111.3 (2)
C-2/C-3/C-4	113.4 (4)	115.4 (2)	C-2/C-3/O-28	108.7 (3)	109.3 (2)
C-4/C-3/O-28	108.3 (3)	108.4 (2)	C-3/C-4/C-5	106.8 (3)	106.5 (2)
C-3/C-4/C-32	111.1 (4)	108.5 (2)	C-5/C-4/C-32	114.0 (4)	108.8 (2)
C-3/C-4/C-33	108.0 (4)	111.5 (2)	C-5/C-4/C-33	109.8 (3)	114.1 (2)
C-32/C-4/C-33	107.1 (4)	107.3 (2)	C-4/C-5/C-6	113.9 (3)	114.9 (2)
C-4/C-5/C-10	118.7 (3)	117.5 (2)	C-6/C-5/C-10	109.4 (4)	109.5 (2)
C-5/C-6/C-7	109.7 (4)	108.4 (2)	C-6/C-7/C-8	116.1 (4)	112.8 (2)
C-7/C-8/C-9	122.1 (4)	123.6 (2)	C-7/C-8/C-14	118.1 (3)	115.5 (2)
C-9/C-8/C-14	119.7 (4)	120.8 (2)	C-8/C-9/C-10	122.0 (4)	122.6 (2)
C-8/C-9/C-11	120.0 (4)	121.6 (2)	C-10/C-9/C-11	117.4 (3)	115.8 (2)
C-1/C-10/C-5	106.8 (3)	107.7 (2)	C-1/C-10/C-9	111.2 (3)	110.0 (2)
C-5/C-10/C-9	109.1 (3)	107.8 (2)	C-1/C-10/C-19	109.3 (3)	108.3 (2)
C-5/C-10/C-19	113.2 (3)	115.3 (2)	C-9/C-10/C-19	107.3 (4)	107.7 (2)
C-9/C-11/C-12	119.2 (4)	116.8 (2)	C-11/C-12/C-13	112.7 (4)	110.5 (2)
C-12/C-13/C-14	106.9 (3)	107.3 (2)	C-12/C-13/C-17	118.7 (3)	119.8 (2)
C-14/C-13/C-17	101.0 (3)	101.4 (2)	C-12/C-13/C-18	109.6 (3)	108.3 (2)
C-14/C-13/C-18	110.6 (3)	111.3 (2)	C-17/C-13/C-18	109.5 (3)	108.5 (2)
C-8/C-14/C-13	112.1 (3)	112.0 (2)	C-8/C-14/C-15	118.5 (4)	117.6 (2)
C-13/C-14/C-15	100.8 (3)	101.3 (2)	C-8/C-14/C-34	104.5 (3)	106.3 (2)
C-13/C-14/C-34	113.1 (3)	112.9 (2)	C-15/C-14/C-34	108.2 (4)	106.9 (2)
C-14/C-15/C-16	104.5 (4)	104.5 (2)	C-15/C-16/C-17	107.1 (4)	107.6 (2)
C-13/C-17/C-16	102.9 (3)	102.1 (2)	C-13/C-17/C-20	119.5 (3)	119.6 (2)
C-16/C-17/C-20	110.6 (3)	113.1 (2)	C-17/C-20/C-21	110.5 (4)	111.8 (2)
C-17/C-20/C-22	116.5 (4)	110.0 (2)	C-21/C-20/C-22	110.1 (4)	110.8 (2)
C-20/C-22/C-23	116.3 (4)	115.7 (2)	C-22/C-23/C-24	114.0 (5)	113.0 (3)
C-23/C-24/C-25	128.4 (5)	128.4 (3)	C-24/C-25/C-26	122.0 (5)	124.0 (3)
C-24/C-25/C-27	124.2 (5)	121.4 (3)	C-26/C-25/C-27	113.8 (5)	114.6 (3)
C-3/O-28/C-29	117.7 (4)	119.7 (3)	O-28/C-29/O-30	122.9 (5)	123.0 (3)
O-28/C-29/C-31	111.7 (5)	110.6 (3)	O-30/C-29/C-31	125.3 (5)	126.4 (3)

Numbers in parentheses are estimated SD.

also examined as the acetate. The mass, ^1H, and ^{13}C NMR spectra of the compounds as well as their chromatographic behavior agreed with the available literature (22–27).

Crystal Data. Euphyl acetate, $C_{32}H_{52}O_2$; M = 468.9; orthorhombic, space group $P2_12_12_1$; **a** = 7.321(3); **b** = 16.641(8); **c** = 23.971(6) Å; V = 2920.5 Å^3; Z = 4; D_c = 1.07 g·cm^{-3}; $F(000)$ = 1040; μ = 4.9 cm^{-1}. Approximate specimen size, 0.15 × 0.15 × 0.25 mm.

Tirucallyl acetate, $C_{32}H_{52}O_2$; M = 468.9; monoclinic, space group $P2_1$; **a** = 8.649(2); **b** = 7.479(1); **c** = 22.774(6) Å; $<\beta$ = 99.24(2)°, V = 1454.0 Å^3; Z = 2; D_c = 1.07 g·cm^{-3}; $F(000)$ = 520; μ = 4.9 cm^{-1}. Approximate specimen size, 0.15 × 0.15 × 0.20 mm.

Structure Determination. The crystals of both euphyl acetate and tirucallyl acetate were clear, colorless, elongated prisms. Intensity data were measured on a Nicolet R3 automatic diffractometer with monochromated CuKα radiation (λ = 1.5418 Å) by the $\theta - 2\theta$ scan technique at room temperature. Additional details about the method of data collection and reduction have been described (28). One quadrant of the reciprocal sphere having $3° \leq 2\theta \leq 114°$ and Friedel-pair reflections in the range of $3° \leq 2\theta \leq 40°$ were measured. A total of 3679 independent reflections were observed for euphyl acetate, of which 2854 were significant; 2254 independent reflections were measured for tirucallyl acetate, of which 2052 were significant. In both analyses the significant

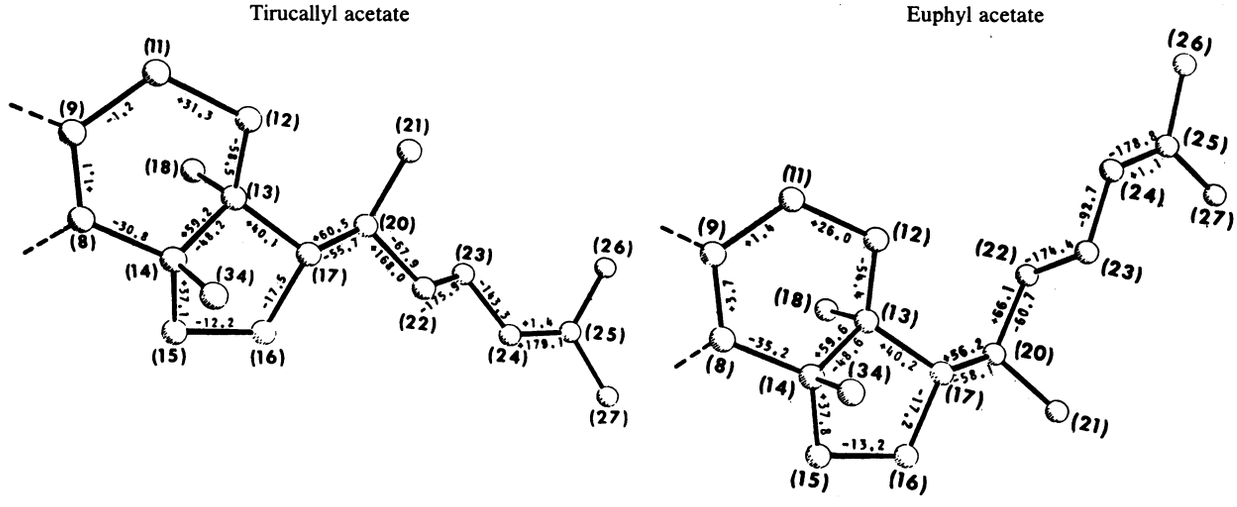

FIG. 3. Crystal structures of tirucallyl and euphyl acetates showing the torsional angles for rings C, D, and the side chain.

unique observed reflections had $|F_o| \geq 2\sigma|F_o|$ and were applied in the data refinement calculation. Intensity data were corrected for background and Lorentz and polarization effects but not for absorption. Both crystal structures were solved by direct methods. Positional and thermal parameters were least-squares refined by a cascade-matrix procedure. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$ with the weighting scheme $w = [\sigma^2(F_o) + 0.001|F_o|^2]^{-1}$. The positions of hydrogen atoms in euphyl acetate were calculated and included in the structure refinement with restriction on their positional parameters. Whereas in tirucallyl acetate, all hydrogen positions were located from a difference-Fourier synthesis. Scattering factors for oxygen were corrected for anomalous dispersion. After the final cycle of refinement, the average parameters shift is 0.12δ for euphyl acetate and 0.05δ for tirucallyl acetate. The agreement index for euphyl acetate is $R = 0.063$ and $R_w = 0.076$; for tirucallyl acetate, $R = 0.034$ and $R_w = 0.044$, where $R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$ and $R_w = \Sigma w(|F_o| - |F_c|)^2/\Sigma wF_o^2$. Final difference-Fourier synthesis showed a residual electron density within ± 0.3 e $Å^{-3}$ for euphyl acetate and ± 0.1 e $Å^{-3}$ for tirucallyl acetate.

Numbering System. The numbering system for the atoms in the two molecules is shown in Fig. 2. For carbon atoms that are common to cholesterol, the numbers are the same as in the steroid system. For other atoms, an arbitrary system was used in the x-ray investigation that incorporates both carbon and oxygen atoms.

Supplementary Materials. Additional tables giving the anisotropic temperature factors for non-H-atoms, the isotropic temperature factors of the hydrogen coordinates, observed and calculated structure factors, torsional angles, and dihedral angles for each of the compounds may be obtained from the authors.

RESULTS AND DISCUSSION

The atomic coordinates, bond lengths, and bond angles for euphyl and tirucallyl acetates are shown in Tables 1, 2, and 3, respectively. The torsional angles for rings C and D and the side chain are shown in Fig. 3. The complete structures of the molecules are given with thermal ellipsoids for the positions of the carbon atoms in Fig. 2, and stereoscopic presentations are to be found in Fig. 4. It will be seen that euphyl acetate is left-handed and tirucallyl acetate is right-handed in the sense that C-22 is *cis*- or *trans*-oriented, respectively, with respect to C-13 around the 17(20)-bond.

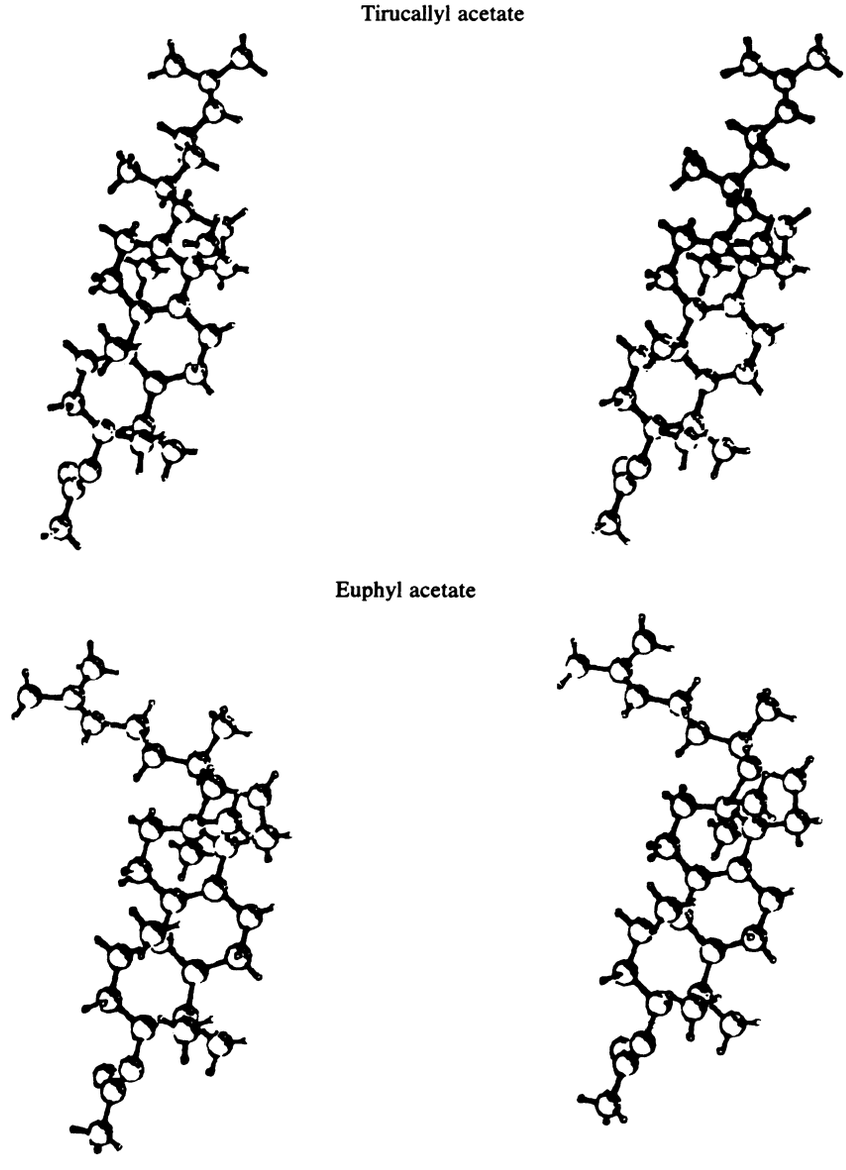

FIG. 4. Stereoscopic drawings of the crystal structures of tirucallyl and euphyl acetates.

Quantitatively, the C(13)-C(17)-C(20)-C(22) torsional angle in tirucallyl acetate is −176.0°, corresponding to a nearly exact *trans*-arrangement. The converse is true for euphyl acetate in which C-21 now almost precisely takes the place of C-22. The C(13)-C(17)-C(20)-C(21) torsional angle (in euphyl acetate) is −177.2°, and C-22 has become *cis*-oriented to C-13. Although the rotomeric condition of C-20 with respect to C-17 can be influenced strongly by nuclear changes (29), the observed rotational isomerism was clearly associated with the configuration at C-20, because inversion of this carbon atom was the only difference between the two molecules studied. The important question (4, 5, 12, 30)—whether similar isomerism exists in the liquid and gaseous states—remains unsettled. It is interesting, however, that Vanderah and Djerassi (31) and Itoh *et al.* (22) have been able to rationalize differences in the spectral and chromatographic properties of C-20 epimers on the assumption that rotational isomerism of the sort shown here actually does occur.

Another interesting aspect of the geometry observed in the present work is that simultaneous inversion of C-13 and C-14 (compared to the steroids) should force ring C to flip so that C-13 is on the back (α) instead of the front (β) face of the molecule. This is necessary for a diequatorial arrangement of C-15 and C-17 to occur, which in turn is required to close ring D. The flip of C-13 with an associated movement of C-14 also forces ring D and the side chain to occupy positions pointing downward onto the back side of the molecule. This can be seen from the torsional angles of Fig. 3 and in a direct visual manner from the stereoscopic drawings of Fig. 4. The bending of the molecule may be the reason why euphoids are not demethylated at C-4 and C-14 and used commonly, as are steroids with a much flatter nucleus, for incorporation into membranes. 9(19)-Cyclosteroids, also bent, similarly appear only to be intermediates and not functional end-products in the steroid pathway (14).

We thank Mr. Marshall E. Benjamin for his careful work on very slow crystallization of 20-epicholesterol. Support of Grant AM-12172 from the National Institutes of Health is gratefully acknowledged.

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