Titration of Sterol Double Bonds with Dibromopyridine Sulfate[1]

FUMIKO U. ROSENSTEIN, RAMON CARUSO, and HENRY W. KIRCHER,
Department of Nutrition and Food Science, The University of Arizona, Tucson, Arizona 85721

ABSTRACT

Cis and *trans*-22-dehydrocholesteryl acetates and *cis* and *trans*-22-cholesten-3β-yl acetates were prepared and compared to Δ^{22}-phytosteryl acetates by titration with dibromopyridine sulfate. The cholesterol derivatives absorbed close to the theoretical quantity of bromine (1 Br_2 per double bond), whereas the Δ^{22}-C_{24}-alkylated sterols consumed 0.14 to 0.23 Br_2 in excess of the calculated values. This excess is attributed to the formation of additional unsaturation during bromination. Δ^7 and $\Delta^{8(14)}$-sterols consume more than 2 and 3 moles Br_2, respectively, which indicates that at least one or two new double bonds are formed in these molecules during the bromination step.

INTRODUCTION

In recent papers, we described the titration of various sterols with dibromopyridine sulfate (1,2). Δ^5-Sterols such as cholesterol, sitosterol, dihydrobrassicasterol and their esters absorbed 1 Br_2 per mole, and stigmastanol used none, as expected. The $\Delta^{5,22}$-sterols, stigmasterol, brassicasterol, and their esters, consistently absorbed 2.15-2.20 Br_2 per mole. We attributed this to the anomalous behavior of the *trans*-Δ^{22} double bond in the side chain of these compounds. In this paper, we present results obtained with some other sterols, notably *cis* and *trans*-Δ^{22}-cholesterol derivatives.

EXPERIMENTAL PROCEDURES

General Methods

Optical rotations were measured with Perkin-Elmer 141 and Rudolph DP 0801 instruments. Melting points are corrected. The ratios of *cis* to *trans* in mixtures of 22-dehydrocholesteryl acetates were estimated by IR (970 cm^{-1}) and thin layer chromatography (TLC) (10% $AgNO_3$-silica gel, 50:50:1 $CHCl_3$-CCl_4-HOAc). Bromine titrations were performed at 2-3 C as described (1) except where noted in Table I. P.e. is petroleum ether, b.p. 65-7 C (Skelly solve B).

Sterols

The acetates of sitosterol (1), brassicasterol (2), dihydrobrassicasterol (2), 7-stigmasten-3β-ol (3), 7,22-stigmastadien-3β-ol (3) and 7,9(11)-ergostadien-3β-yl benzoate (4) were available from earlier work. The acetates of cholesterol, stigmasterol, 7-cholesten-3β-ol, 7-ergosten-3β-ol, 7,22-ergostadien-3β-ol, and 8(14)-ergosten-3β-ol were prepared by conventional procedures (5). 5β-22-Stigmasten-3β-ol was a gift of H.J. Eyssen (6). Poriferasteryl acetate was prepared by the methods described by Sucrow et al. m.p. 148 C, $[\alpha]_D$-55.5°, lit (7) m.p. 146-8 C, $[\alpha]_D$-52.2°.

3β-Acetoxy-bisnor-5-cholen-22-al (2)

The aldehyde was prepared by modifications of McMorris' procedures (8). 3β-Acetoxy-bisnor-5-cholen-22-oic acid (1, 50 g, Steraloids, Inc. Wilton, NH) 100 ml thionyl chloride and 500 ml benzene were refluxed 1 hr, cooled and evaporated to dryness in vacuo. The residue was refluxed 1 hr with 100 g imidazole in 500 ml benzene, allowed to stand overnight, and the two phases evaporated to dryness. The residue was extracted with 1 liter. methanol and dried to yield 50 g imidazolid, m.p. 223.5-7 C, lit (8) 220-3 C. This was refluxed under N_2 in 700 ml tetrahydrofuran (THF) and reduced by the addition of 46 g LiAlH $(OtBu)_3$ in 500 ml THF to yield 43 g of an 80:20 mixture (gas liquid chromatography, GLC) of 2 and 3β-acetoxy-bisnor-5-cholen-22-ol (3) after conventional workup (8). Comparable reductions at room temperature gave lower yields of aldehyde. Separation of 2 and 3 was feasible (8), but not necessary for the subsequent Witting reaction; 2 m.p. 119-120.5 C (from acetone and p.e.), lit (8) 113-6 C, 3 m.p. 156-7.5 C (from acetone and ether), lit (9) 153-4 C.

5,22Z- and 5,22E- Cholestadien-3β-ylAcetates (4 and 5, respectively)

A solution of 320 g triphenylphosphine and 220 ml 1-bromo-3-methylbutane in 1500 ml xylene was refluxed 4 days. Solvent was decanted from the cooled mixture and the residue refluxed with dry THF containing a

[1]Arizona Agricultural Experiment Station Journal Article No. 2664.

TABLE I

Bromine Absorption by Sterol Double Bonds

Sterol type	Acetates of:	Br_2 per mole
Stanol	Bisnor-5α-cholan-22-oic acid	0.004,0.004
5-Monoene	Cholesterol	0.99,1.00
	Sitosterol	1.01,1.02
	Bisnor-5-cholen-22-oic acid	0.98,0.99
22-Monoene	22Z-Cholesten-3β-ol	1.02,1.03
	22E-Cholesten-3β-ol	1.03,1.04
	5β-22E-Stigmasten-3β-ol[1]	1.22,1.23
5,22-Diene	5,22Z-Cholestadien-3β-ol	2.01,2.01
	5,22E-Cholestadien-3β-ol	2.01,2.01
	Brassicasterol	2.17,2.19
	Poriferasterol	2.14,2.15
	Stigmasterol	2.17,2.20
		2.16[b],2.26[c]
7-Monoene	7-Cholesten-3β-ol	2.12,2.12
	7-Ergosten-3β-ol	2.13,2.16
	7-Stigmasten-3β-ol	2.03,2.09
7,9(11)-Diene	7,9(11)-Ergostadien-3β-ol[d]	2.08,2.10
7,22-Diene	7,22-Ergostadien-3β-ol	3,14,3.16
	7,22-Stigmastadien-3β-ol	2.99,3.01
8(14)-Monoene	8(14)-Ergosten-3β-ol	3.08,3.22

[a]Free sterol titrated
[b]−18 C
[c]28 C
[d]Benzoate titrated

little absolute ethanol. The salt was obtained on cooling in 53% yield, m.p. 154-6 C, lit (10) 158-9 C.

Butyl lithium (30 ml, 1.6 M in hexane) was added to a suspension of 21.9 g triphenyl-3-methylbutyl-phosphonium bromide in 300 ml dry p.e. and the mixture refluxed 5 min under N_2. A suspension of the **2-3** mixture (9 g) in 250 ml dry p.e. was quickly added to the hot solution and the resulting mixture stirred at 40-60 C 1 hr and at room temp overnight. The reaction was poured into water, the organic layer evaporated, the residue acetylated and crystallized from methanol to yield 5-6 g of a 25:75 mixture of **4** and **5**. The two were separated on 20% silver nitrate-silica gel columns (5 g/kg adsorbent) with 10:1 hexane-benzene and recrystallized from methanol: **4***(cis)* m.p. 116-7 C, $[\alpha]_D$-69.5°, lit (10) m.p. 115-6 C, $[\alpha]_D$-68°; **5**(*trans*) m.p. 128-9 C, $[\alpha]_D$-60.7' lit (10) m.p. 125-8 C, $[\alpha]_D$-61°. The methyl group nuclear magnetic resonances (NMR) of **4** and **5** corresponded to published values (10,11); in addition, there were significant differences in the absorptions of the side chain vinyl protons (Fig. 1).

5α-22Z- and 5α-22E-Cholesten-3β-yl Acetates (6 and 7, respectively)

Acid **1** was reduced with H_2-10% Pd/C-ethyl acetate to 3β-acetoxy-bisnor-5α-cholan-22-oic acid [m.p. 194-5.5 C, lit (12) 194 C], which was transformed by the methods described above to the *cis* and *trans* 5α-22-cholestenyl acetates: **6***(cis)* m.p. 104-5.5 C $[\alpha]_D$-20.8°; **7***(trans)* m.p. 105-6.5 C, $[\alpha]_D$-7.7°, lit (13) m.p. 104-5 C. The NMR spectra of the vinyl protons in the two compounds (Fig. 1) are analogous to those of **4** and **5** except for the absence of absorption at 5.37 (C_6 hydrogen) in **6** and **7**.

RESULTS AND DISCUSSION

The absorption of bromine from dibromo pyridine sulfate by the double bonds of various sterols is shown in Table I. Our earlier results (1,2) were confirmed; Δ^5 sterols consumed 1.00 ± 0.02 Br_2 per mole and $\Delta^{5,22}$ phytosterols 2.17 ± 0.03 Br_2 per mole. The influence of temperature on this overconsumption was small (Table I, stigmasteryl acetate). The data also show how substitution at C_{24} influences

bromine absorption. The four Δ^{22} and $\Delta^{5,22}$ compounds alkylated at C_{24} all consumed 0.14 to 0.23 moles of Br_2 in excess of calculated values, whereas the corresponding cholesterol derivatives (Δ^{22} *cis* or *trans*) consumed only 0.01 to 0.04 moles of excess Br_2 per mole of sterol.

The principal difference between these two classes of compounds which might explain this is the presence of an extra tertiary hydrogen atom, allylic to the double bond in C_{24}-alkyl sterols, which could participate in the development of some additional unsaturation during the bromination (Fig. 2A). The absence of a C_{24} tertiary hydrogen atom would then be the reason why Δ^{22} cholesterol derivatives (Fig. 2B) absorb nearly the theoretical amount of Br_2 per mole.

There are some justifications for this hypothesis. The loss of the proton on C_{25} from an intermediate carbonium ion to form a $\Delta^{24(25)}$ double bond is suggested to occur during phytosterol biosynthesis (14). In addition, the recovery of stigmasteryl acetate from its insoluble tetrabromide with zinc is only 78% (2) while the recovery of cholesterol from its 5,6-dibromide is at least 93% (15). This suggests that during the bromination of stigmasteryl acetate, compounds other than the 5,6;22,23-tetrabromide are formed.

Bromination of sterol double bonds in other

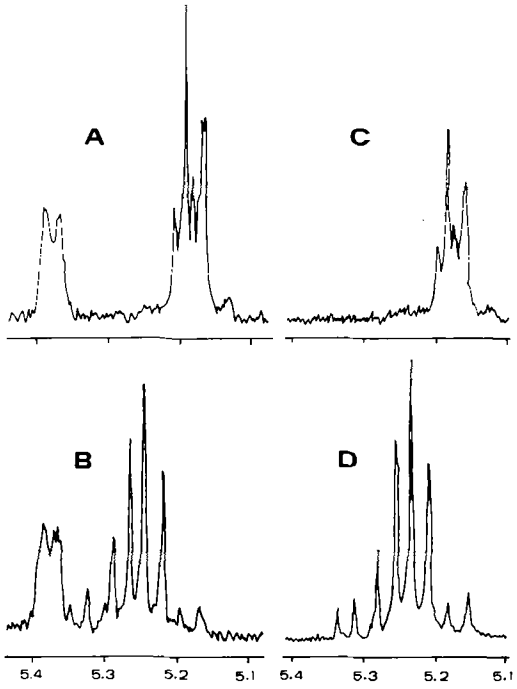

FIG. 1. Sections of the 270 MHZ spectra of compounds 4(A), 5(B), 6(C), and 7(D) showing the absorptions in the vinyl proton region. C_6 proton: δ = 5.37 (doublet); C_{22} and C_{23} protons: δ = 5.15 to 5.22 (multiplet) for the *cis*-Δ^{22} double bond. δ = 5.15 to 5.34 (octet) for the *trans*-Δ^{22} double bond.

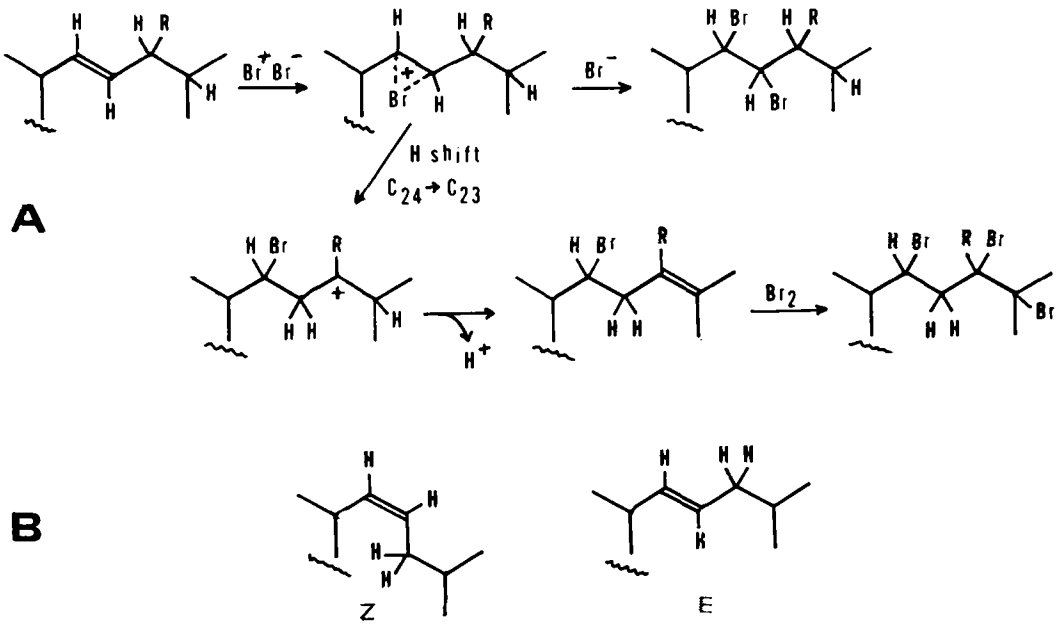

FIG. 2. A-Possible bromination pathways of C_{24}-alkylated (R = methyl or ethyl) sterols. B-Side chain of Δ^{22}-unsaturated cholesterols: Z = *cis*, E = *trans*.

positions gave widely varying results. Δ^7-Sterols consumed more than 2 Br_2 per mole, so an additional double bond must form during the bromination step. Zinc debromination of the reaction product followed by TLC and UV analysis showed the formation of 7,9(11)- and other dienes in these cases. Authentic 7,9(11)-ergostadienyl benzoate, however, absorbed only a small excess of Br_2 over the theoretical value of 2 Br_2 per mole.

During the bromination of 8(14)-ergostenyl acetate, two new double bonds must have formed to account for the 3+ Br_2 consumed per mole of this sterol. TLC of the products after zinc debromination showed complete absence of starting material with numerous very low R_f spots.

The analysis of sterols by this method of titration is, therefore, valid for stanols (0 Br_2/mole), Δ^5-stenols (1 Br_2/mole) and Δ^{22} or $\Delta^{5,22}$-cholesterol derivatives. The sterols we tested that have alkylation at C_{24} allylic to the Δ^{22} double bond or unsaturation at Δ^7 or $\Delta^{8(14)}$ consumed bromine in excess of the calculated values.

The Δ^{22}-cholestenols have not been prepared before, although the *trans* compound was reported once as a constituent of sponges (13). Our synthesis paralled that of the known $\Delta^{5,22}$ derivatives and the spectroscopic and chromatographic properties of the 22Z and 22E isomers were comparable to those of the 22-dehydrocholesterols.

ACKNOWLEDGMENTS

We wish to thank M. Zeisberg (Berlin, Federal Republic Germany) for 270 MHZ NMR spectra. This work was supported in part by NSF Grant DEB 74-19148 A03.

REFERENCES

1. Rosenstein, F.U., and H.W. Kircher, Lipids 8:107 (1973).
2. Kircher, H.W., and F.U. Rosenstein, Ibid, 8:453 (1973).
3. Kircher, H.W., and F.U. Rosenstein. J. Org. Chem. 35:2259 (1973).
4. Kircher, H.W., and F.U. Rosenstein, Lipids 10:517 (1975).
5. Fieser, L.F., and M. Fieser, "Steroids," Reinhold, New York, N.Y. 1959.
6. Eyssen, H.J., G.G. Parmentier, F.C. Compernolle, G. Depauw, and M. Piessons–Denef, Eur. J. Biochem. 36:411 (1973).
7. Sucrow, W., P. Polyzou Caldeira, and M. Slopianka, Chem. Ber. 106:2236 (1973).
8. McMorris, T.C., J. Org. Chem. 35:458 (1970).
9. Hayazu, R., Pharm. Bull. (Tokyo) 5:452 (1957).
10. Hutchins, R.F.N., M.J. Thompson, and J.A. Svoboda, Steroids 15:113 (1970).
11. Rubinstein, I., L.J. Goad, A.D.H. Clague, and L.J. Mulheirn, Phytochemistry 15:195 (1976).
12. Fernholz, E., Ann. 507:128 (1933).
13. Erdman, T.R., and R.H. Thompson, Tetrahedron 28:5163 (1972).
14. Lenton, J.R., L.J. Goad, and T.W. Goodwin, Phytochemistry 14:1523 (1975).
15. Fieser, L.F., J. Am. Chem. Soc. 75:5421 (1953).

[Received October 12, 1976]