361. *Studies in the Sterol Group. Part XLVIII. 7-Substituted Cholesterol Derivatives and their Stereochemistry (Part I). 7-Halogeno-derivatives.*

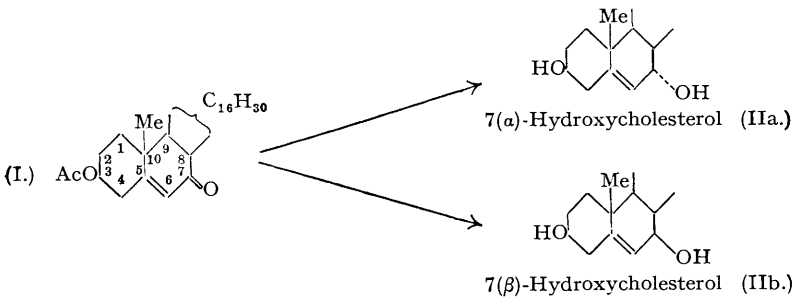
By A. E. Bide, H. B. Henbest, E. R. H. Jones, and P. A. Wilkinson.

The stereochemistry of 7-substituted cholesterol derivatives is reviewed. Attention is drawn to the unusually large optical rotation differences observed between epimerides in the 7-position and it is suggested that the sign of rotation provides a definite indication of their relative stereochemical configuration. The absolute configuration of these 7-substituted cholesterol derivatives is discussed.

In the preceding paper the preparation of "β"-7-bromocholesteryl esters by bromination of cholesteryl esters with *N*-bromosuccinimide has been described and for comparison purposes a number of 7-halogeno-compounds have now been made from the corresponding 7-hydroxy-compounds by employing thionyl chloride or phosphorus halides. It appears that the 7-halogeno-compounds with the "β"-configuration are inherently more stable than the "α"-epimerides.

Much of the work carried out hitherto on 7-substituted cholesterol derivatives has involved the use, as starting material, of the readily accessible 7-ketocholesteryl acetate (I), first obtained by

Mauthner and Suida (*Monatsh.*, 1896, **17**, 593) by oxidation of cholesteryl acetate with chromic acid, this preparation being subsequently improved by Windaus, Lettré, and Schenck (*Annalen*, 1935, **520**, 98). The latter authors reduced this keto-acetate with aluminium *iso*propoxide and *iso*propyl alcohol and obtained, after hydrolysis, one of the two possible 7-hydroxycholesterols (IIa or IIb), m. p. 178°. The second isomer, m. p. 186°, was subsequently obtained in small



yield by Barr, Heilbron, Parry, and Spring (*J.*, 1936, 1437) by oxidising cholesteryl hydrogen phthalate with potassium permanganate, followed by hydrolysis. A more recent and more detailed study of the Meerwein–Ponndorf reduction of the keto-acetate (I) by Wintersteiner and Ruigh (*J. Amer. Chem. Soc.*, 1942, **64**, 1177, 2453) has revealed, as would be expected, that the epimeric 7-hydroxy-compounds are in fact produced simultaneously, the second form (m. p. 186°), however, only in minor proportion. Ruzicka, Prelog, and Tagmann (*Helv. Chim. Acta*, 1944, **27**, 1149) have shown that an equilibrium is set up when either of these diols is refluxed in acetic acid solution, the equilibrium mixture containing about 65% of the Windaus diol.

The convention now generally adopted for representing the stereochemical configuration of substituted steroids, namely, by suffixing with (α) or (β) the number of the nuclear carbon atom carrying the substituent, has been briefly described in the preceding paper. An excellent detailed account has been given recently by Shoppee (*Ann. Reports*, 1946, **43**, 200). Windaus *et al.* (*loc. cit.*) had no knowledge of the configuration of their diol (m. p. 178°) relative to the C_{10} methyl group, but rather unfortunately they designated it 7(α)-hydroxycholesterol. On the other hand, Heilbron *et al.* (*loc. cit.*), recognising that the true configuration of the 7-hydroxyl group in their diol was unknown, termed the latter β-7-hydroxycholesterol. In the present series of papers the nomenclature " α "-7- and " β "-7-hydroxycholesterols is used for the above diols and for their derivatives; in this way not only is the absence of information concerning orientation relative to the C_{10} methyl group made clear, but relationships to the original epimeric diols can thus be indicated.

Comparison of the optical rotations of the epimeric 7-hydroxycholesterols and their simple esters (Table I) reveals that the " α "-diol and its derivatives are uniformly dextrorotatory whereas the compounds of the " β "-group are lævorotatory :

TABLE I.

" α "-Series.	$[\alpha]_D$ in $CHCl_3$.	" β "-Series.	$[\alpha]_D$ in $CHCl_3$.
" α "-7-Hydroxycholesterol	+ 7·2° [1]	" β "-7-Hydroxycholesterol	− 86·4° [4]
	+ 4° [2]		− 87·6° [1]
,, diacetate	+51·8° [1]	,, diacetate	−174·6° [4]
,, dibenzoate	+94·3° [3]		−177° [2]
	+94° [2]	,, dibenzoate	−107·5° [1]

[1] Wintersteiner and Ruigh, *loc. cit.*, p. 2453.
[2] Ruzicka, Prelog, and Tagmann, *loc. cit.*
[3] Windaus, Lettré, and Schenck, *loc. cit.*
[4] Barr, Heilbron, Parry, and Spring, *loc. cit.*

The large differences between these epimeric forms [even larger differences are observed with the epimeric 7-amino-compounds and also with their *N*-acetyl derivatives (Barnett, Ryman, and Smith, *J.*, 1946, 524; Eckhardt, *Ber.*, 1938, **71**, 469)] are unusual in the steroid series, but they have been amply confirmed and extended by the values for a number of pairs of new 7-substituted cholesterol derivatives prepared during the present study and described in this and the following papers. The sign of optical rotation can only rarely be employed as an indication of configuration, but the differences here are so marked that it can safely be

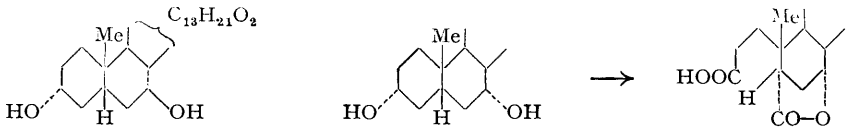
postulated as a general rule that " α "-7-substituted cholesterol derivatives are dextrorotatory while the " β "-7-compounds are lævorotatory. [A list of the 7-derivatives now known, together with their optical rotations, is given in the Table in Part L, this vol., p. 1798.]

The marked differences in optical rotatory properties between epimeric 7-substituted cholesterol derivatives must be connected with some " vicinal action " (cf. Barton and Jones, *J.*, 1944, 659; Barton, *J.*, 1945, 813; 1946, 512; Barton and Cox, *Nature*, 1947, **159**, 470) of the 5 : 6-double bond, since much smaller differences (see Table II) are observed between the epimeric dihydro-compounds [prepared by Wintersteiner and Moore (*J. Amer. Chem. Soc.*, 1943, **65**, 1503) by the catalytic hydrogenation of 7-ketocholestanol], although even these are large

TABLE II.

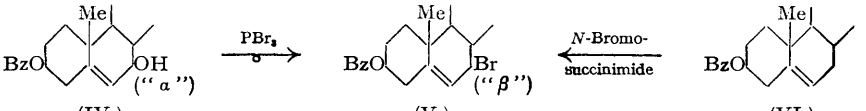
	Cholestane $[\alpha]_D$ in $CHCl_3$.	Cholest-5-ene $[\alpha]_D$ in $CHCl_3$.
" α "-7-3(β)-Diol	+53°	+ 7°
,, diacetate	+55°	+ 52°
" β "-7-3(β)-Diol	+ 8°	− 87°
,, diacetate	−17°	−175°

when compared with the values for epimers in the 3-position (Callow and Young, *Proc. Roy. Soc.*, 1936, **157**, *A*, 194). The " vicinal action " may take the form of deflection of valency angles in one or both of the 7-epimers as a result of steric factors. It seems highly probable that the two series of 7-hydroxycholestanol derivatives are related to their 7-hydroxycholesterol analogues in the way that the signs of their optical rotation suggest (Wintersteiner and Moore, *loc. cit.*), but confirmation of this by hydrogenation of the cholest-5-ene compounds is unfortunately impossible since hydrogenation results in removal of the 7-substituent in all the instances so far studied (Barr, Heilbron, Parry, and Spring, *loc. cit.*; Wintersteiner and Ruigh, *loc. cit.*; see also Part L, *loc. cit.*).



(IIIa.) (IIIb.)

In the bile-acid series the configurations of the 7-hydroxyl groups are known as a result of oxidative degradation and lactone formation experiments (Windaus and von Schoor, *Z. physiol. Chem.*, 1925, **148**, 225; Iwasaki, *ibid.*, 1936, **244**, 181); thus chenodeoxycholic acid (IIIb) contains a 7(α)-hydroxyl group whereas the 7-hydroxyl group in its epimer, ursodeoxycholic acid (IIIa), is (β)-orientated. If it can be assumed that the structural differences between the hydrogenated sterol and the bile acid series, *i.e.*, in the side chains and more particularly at C_5, do not exert any appreciable effect on molecular rotation differences between epimeric pairs of 7-substituted compounds, then the conclusions of Plattner and Heusser (*Helv. Chim. Acta*, 1944, **27**, 748), based on these differences [+ 175° (in alcohol) between cheno- and urso-deoxycholic acids and − 181° (in chloroform) between the " α "-7- and " β "-7-hydroxycholestanols], lead to the determination of configuration in these hydroxycholestanols. The arbitrary assignments " α "-7- and " β "-7- used in these papers, and in various forms by other workers, would thus need to be reversed in order to designate the configuration relative to the C_{10} methyl group. However, as has been pointed out by Shoppee (*Ann. Reports, loc. cit.*), the further extension of these assumptions to the 7-hydroxycholesterols is at variance with conclusions based upon the relative ease of elimination of benzoic acid from isomeric 7-benzoyloxycholesterol derivatives (Wintersteiner and Ruigh, *loc. cit.*), and, although it is conceivable that the usually facile *trans*-elimination may be swamped by steric factors, some more rigid proof of the configurations of the 7-substituted sterol derivatives is highly desirable.

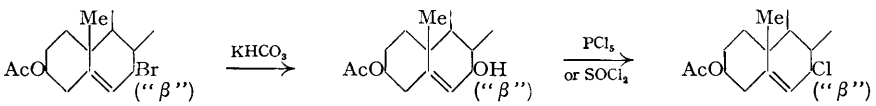


(IV.) (V.) (VI.)

In the preceding paper preparations of monobromo-compounds by the action of *N*-bromo-succinimide on cholesteryl acetate, benzoate, chloride, and bromide are described, and these

compounds have all been formulated as belonging to the " β "-7- series. In order to accumulate evidence in support of these formulations the preparation of epimeric 7-halogeno-derivatives by alternative routes has been studied. " α "-7-Hydroxycholesteryl benzoate (IV), prepared by Eckhardt (*loc. cit.*) by the partial benzoylation of the Windaus " α "-7-hydroxycholesterol, was clearly the most convenient starting material for this purpose. Wintersteiner and Moore (*loc. cit.*) had reported that the saturated 7-hydroxycholestanyl esters did not readily yield 7-halogeno-derivatives, but fortunately the behaviour of (IV) was found to be in sharp contrast to these observations. Treatment of the dextrorotatory mono-benzoate (IV) with phosphorus tribromide in ethereal suspension yielded " β "-7-bromocholesteryl benzoate (V), identical with the product obtained (preceding paper) by the reaction of cholesteryl benzoate (VI) with *N*-bromosuccinimide. Similarly, with phosphorus pentachloride the strongly laevorotatory " β "-7-*chlorocholesteryl benzoate* was produced. It seems quite clear that stereochemical inversions must be involved in these substitution reactions with the phosphorus halides. Experiments in which phosphorus trichloride or thionyl chloride was employed yielded non-homogeneous products which, according to their specific rotations, appeared to be mixtures of the epimeric " α "- and " β "-7-chloro-compounds. However, by treating a pyridine solution of the monobenzoate (IV) with thionyl chloride at 0° it was possible to isolate the dextrorotatory " α "-epimer, " α "-7-*chlorocholesteryl benzoate*, as the major product of the reaction.

From a study of the mild alkaline hydrolysis of " β "-7-bromocholesteryl acetate (VII), the corresponding lævorotatory " β "-7-hydroxycholesteryl acetate (VIII) became available (described in Part XLIX). Treatment of this monoacetate with either thionyl chloride



(VII.) (VIII.) (IX.)

or phosphorus pentachloride readily gave " β "-7-*chlorocholesteryl acetate* (IX). Thus while phosphorus pentachloride produces substitution with inversion in an " α "-7-hydroxy-compound, and the use of thionyl chloride results in substitution with partial inversion, both reagents effect substitution in the " β "-7-hydroxy-compound without any appreciable inversion of configuration at the 7-position.

These facts, together with the knowledge that bromination with *N*-bromosuccinimide fails to produce any detectable quantities of the " α "-7-bromo-compounds (preceding paper), suggest an inherently greater stability of the 7-halogeno-compounds of the cholesterol series with a " β "-orientation.

The instability of the " β "-7-compounds is apparently only manifest in the transition state in replacement reactions, since treatment of the " α "-7-chloro-benzoate with phosphorus pentachloride fails to effect any isomerisation. It may further be concluded that the difficulty of preparing these " α "-7-compounds, either by replacement from the hydroxy-compounds or by direct bromination with *N*-bromosuccinimide, may result from steric hindrance between the C_{10}(β)-methyl group and the C_7-halogen atom. This would probably be very considerable if the C_7 halogen atoms had the true (β)-configuration, *i.e.*, if they were on the same side of the plane of the steroid molecule as the C_{10}-methyl group. Such a conclusion reinforces the deduction of Plattner and Heusser (*loc. cit.*) that the true C_7 configurations are the reverse of those indicated by the trivial " α " and " β " prefixes.

Treatment of " β "-7-bromocholesteryl acetate (VII) with sodium iodide in acetone gave a quantitative yield of " β "-7-*iodocholesteryl acetate* with the unusually high rotation ($[\alpha]_D^{20°}$) of − 365°. The corresponding *benzoate* was similarly prepared.

The optical rotatory properties of all of the 7-halogeno-cholesteryl esters are collated in the Table in Part L (*loc. cit.*). The identical stereochemical configurations of the " β "-7-chloro-, -bromo-, and -iodo-acetates are strikingly confirmed by their reactions with methylaniline, when all yield the same 7-methylanilino-derivative (forthcoming publication).

The epimeric 7-chloro-benzoates, when treated with diethylaniline at 150°, gave practically identical yields (30%; estimated spectrographically) of 7-dehydrocholesteryl benzoate. It is to be noted that Wintersteiner and Ruigh (*loc. cit.*) found marked differences in the behaviour of the epimeric 7-benzoyloxy-compounds under comparable conditions. The 7-iodo-compounds react with diethylaniline even at room temperature giving products, containing nitrogen but no iodine, the structure of which will be discussed in a subsequent communication.

Experimental.

"β"-7-*Bromocholesteryl Benzoate* (V).—Finely powdered "α"-7-hydroxycholesteryl benzoate (3 g.) suspended in ether (100 c.c.) was treated with phosphorus tribromide (3 g.); the steroid soon went into solution. After 1 hour at room temperature, the product was isolated with ether. Two crystallisations from acetone gave "β"-7-bromocholesteryl benzoate (1·6 g.), m. p. 140° (undepressed on admixture with a sample prepared by the *N*-bromosuccinimide reaction), $[\alpha]_D^{19°}$ − 175° (*c*, 1·05).

"β"-7-*Chlorocholesteryl Benzoate*.—Powered "α"-7-hydroxycholesteryl benzoate (2 g.) (Eckhardt, *loc. cit.*) was added to ether (40 c.c.) and benzene (10 c.c.), followed by phosphorus pentachloride (2 g.) which caused the suspended material to dissolve. After 30 minutes at room temperature, the product was isolated *via* ether and 2 crystallisations from acetone–benzene (4 : 1) gave "β"-7-*chlorocholesteryl benzoate* (1·0 g.), m. p. 149—150°; $[\alpha]_D^{18°}$ − 119° (*c*, 0·52) (Found: C, 77·8; H, 9·4. $C_{34}H_{49}O_2Cl$ requires C, 77·75; H, 9·4%).

"α"-7-*Chlorocholesteryl Benzoate*.—A mixture of thionyl chloride (3 c.c.) and dry pyridine (10 c.c.) was added to a solution of "α"-7-hydroxycholesteryl benzoate (2 g.) in dry pyridine (30 c.c.), both solutions having previously been cooled to 0°. The mixture was kept at 0° for 30 minutes, and was then poured on ice, and extracted with ether. The ethereal extract was washed with dilute acetic acid, water and sodium hydrogen carbonate solution and finally filtered, dried, and evaporated under reduced pressure. The residue was crystallised from acetone–benzene (4 : 1), and recrystallisation from acetone–ethyl acetate (4 : 1) gave "α"-7-*chlorocholesteryl benzoate* (0·44 g.) as plates, m. p. 151°, $[\alpha]_D^{20°}$ + 60·7° (*c*, 0·91) (Found: C, 77·85; H, 9·1%).

"β"-7-*Chlorocholesteryl Acetate* (IX).—Thionyl chloride (0·5 c.c.) was added to a solution of "β"-7-hydroxycholesteryl acetate (500 mg.; see Part XLIX) in dry ether (25 c.c.) at 0° and the mixture was then kept at 20° for 1 hour. Removal of volatile materials under reduced pressure and 2 crystallisations of the residual solid from dry acetone gave "β"-7-*chlorocholesteryl acetate* (290 mg.) as long needles, m. p. 114°, $[\alpha]_D^{20°}$ − 165° (*c*, 0·87) (Found: C, 75·0; H, 10·2. $C_{29}H_{47}O_2Cl$ requires C, 75·2; H, 10·2%).

"β"-7-*Iodocholesteryl Acetate*.—"β"-7-Bromocholesteryl acetate (5 g.) was dissolved in dry acetone (70 c.c.) by gentle warming. When a solution of sodium iodide (5 g.) in dry acetone (30 c.c.) was added with stirring there was an immediate precipitation of sodium bromide, which was filtered off after 1 minute. The filtrate was then cooled in a carbon dioxide–acetone bath and the resulting crystals were filtered off as soon as crystallisation had ceased, since the iodo-compound decomposes slightly in solution, becoming greenish. Any contact of the iodo-compound with metals (*e.g.*, a nickel spatula) should be avoided, since it causes the product to become greenish-black; a glass spatula was used. The pale yellow iodo-compound (4·2 g.) was dried under reduced pressure and had m. p. 91°. Recrystallisation from acetone gave "β"-7-*iodocholesteryl acetate* (3·65 g.), m. p. 92—93°, $[\alpha]_D^{20°}$ − 365° (*c*, 1·02) (Found: C, 63·3; H, 8·5. $C_{29}H_{47}O_2I$ requires C, 62·8; H, 8·55%).

"β"-7-*Iodocholesteryl Benzoate*.—A solution of sodium iodide (0·5 g.) in dry acetone (7 c.c.) was added to a solution of "β"-7-bromocholesteryl benzoate (0·5 g.) in benzene (3 c.c.), and the mixture was swirled around for 2 minutes. The supernatant liquid was decanted from the inorganic precipitate, which was washed with benzene (1 c.c.). Acetone (8 c.c.) was added and the solution cooled to 0° to induce crystallisation and finally cooled in a carbon dioxide–acetone bath to give the crude iodo-compound (0·48 g.), m. p. 108° (decomp.) (when put in bath at 90°). Dissolution in cold benzene, followed by precipitation with acetone, gave "β"-7-*iodocholesteryl benzoate*, m. p. 115° (decomp.) (when put in bath at 110°), $[\alpha]_D^{17°}$ − 270° (*c*, 0·45) (Found: C, 66·85; H, 8·05. $C_{34}H_{49}O_2I$ requires C, 66·2; H, 8·0%).

Imperial College of Science and Technology, London, S.W. 7.
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