phosphorane. Extensive chromatography gave the *cis* isomer, m.p. 125–127°, which on vapor phase analysis exhibited one of the peaks indicated above.

Anal. Calcd. for $C_{15}H_{10}BrNO_3$: C, 54.07; H, 3.33; Br, 23.99; N, 4.21. Found: C, 54.51; H, 3.09; Br, 24.3; N, 4.15.

Repetition of the experiment but analyzing the product following the chromatography indicated: after 10 hr., a 28:72 *trans:cis* ratio; after 20 hr., 26:74; and after 30 hr., 25:75.

B. **From the Tributyl Ylid.**—*p*-Nitrobenzaldehyde (1.5 g., 0.01 mole) was added to a solution of crude 2-bromo-2-(tributylphosphoranylidene)-acetophenone[26] (0.01 mole) in methylene chloride (50 ml.) and let stand 18 hr. The solution was then concentrated, and chromatographed in benzene on Fisher A-540 alumina. The carbonyl-containing eluents (infrared) were combined, concentrated, and recrystallized from methanol to give 0.85 g., m.p. 110–111°. Two recrystallizations from methanol gave m.p. 118–120°. Vapor phase chromatography under conditions shown to separate the *cis* and *trans* isomers gave only one major peak, *i.e.*, the *cis* isomer. A second crop was obtained, 0.53 g., m.p. 67–71°. Recrystallization of this solid from methanol gave a material, m.p. 75.0–75.5. Vapor phase chromatography indicated this to be a *cis–trans* mixture.

(26) This ylid was prepared in methylene chloride by treating a solution of the corresponding phosphonium bromide in methylene chloride with aqueous sodium carbonate. The phosphonium bromide was prepared in 60% yield by bromination of tributylphosphoranylideneacetophenone. *Anal.* Calcd. for $C_{20}H_{33}Br_2OP$: C, 50.01; H, 6.93; Br, 33.28; P, 6.45. Found: C, 49.57; H, 6.90; Br, 32.88; P, 6.58.

Acknowledgment.—We wish to thank Miss Joan E. Fedder for the large-scale preparation of many intermediates and compounds used in this study and Dr. D. E. Bissing for many helpful discussions.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, STEVENS INSTITUTE OF TECHNOLOGY, HOBOKEN, N. J.]

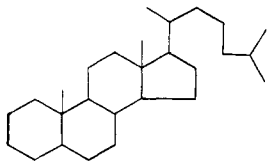
Conformation of Some Terpenoids from Dipole Moment and Nuclear Magnetic Resonance Studies[1]

BY A. K. BOSE, M. S. MANHAS, AND E. R. MALINOWSKI

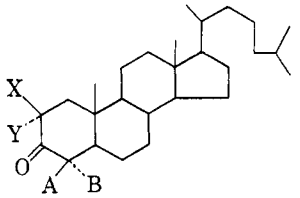
RECEIVED MARCH 16, 1963

The stereochemistry of halogen-substituted cholestan-3-ones and allobetulones has been studied on the bases of dipole moments, proton magnetic resonance, and concepts of conformational analysis. The conformation of ring A is deduced to be very nearly a chair in 2α-bromo- and 2α,4α-dibromocholestan-3-one, distorted chair in allobetulone and 2α-bromoallobetulone, planar in 2,2-chlorobromocholestan-3-one, and planar or boat in 2β-bromo- and 2α,2β-dibromoallobetulone.

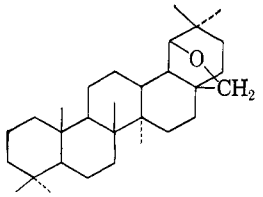
From spectral and optical rotatory dispersion data it has been suggested that certain substituted α-halocyclohexanones may prefer the boat to the chair conformation.[2] Since dipole moment measurements can yield additional information about the conformation of suitably substituted molecules, we undertook a study of the dipole moments of cholestan-3-one (II), allobetulone (VII), several of their halogen derivatives, and some related compounds. Similar work has been reported recently.[3,4]



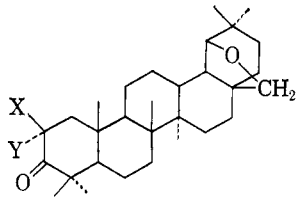
I, cholestane



II, cholestan-3-one, X = Y = A = B = H
III, 2α-bromocholestan-3-one, X = A = B = H, Y = Br
IV, 2α,4α-dibromocholestan-3-one, X = A = H, Y = B = Br
V, 2ζ-chloro,2ζ-bromocholestan-3-one, X or Y = Cl, Y or X = Br, A = B = H



VI, 3-desoxyallobetulone



VII, allobetulone, X = Y = H
VIII, 2α-bromoallobetulone, X = H, Y = Br
IX, 2β-bromoallobetulone, X = Br, Y = H
X, 2α,2β-dibromoallobetulone, X = Y = Br

Another physical method for getting information about the shapes of suitable molecules is proton magnetic resonance spectroscopy. In particular, the vicinal proton–proton spin-spin coupling constant has been shown to be sensitive to changes in the dihedral angle between two carbon–hydrogen bonds.[5,6] This prompted us to include n.m.r. studies in our investigation.

Experimental

Dipole Moment.—The dielectric apparatus used in this study was essentially the same as that designed by Chien.[7] A parallel-plate dielectric cell was constructed especially to accommodate very small samples while maintaining a high degree of precision. The cell had an effective capacitance of 55.85 μμf. and required less than 2 ml. of solution. For each dipole moment measurement approximately 50 to 100 mg. of sample was used. The data listed in Table I were determined from dilute benzene solutions at 25°.

The method of Halverstadt and Kumler,[8] programmed for an IBM 1620 computer, was used to calculate the moments. Molar refractivities were calculated from atomic refractivities for the sodium D line. The experimental error was estimated to be approximately ±0.03 D.

N.m.r. Spectra.—A Varian high resolution DP-60 spectrometer was used for proton magnetic resonance studies. The spectra were measured at 60 Mc./sec. on sample dissolved in deuterated chloroform containing a trace of tetramethylsilane as an internal standard. Calibration of the graphs was accomplished by the usual side-band technique.

(1) Based in part on a paper presented before the Second International Symposium on the Chemistry of Natural Products, Prague, August, 1962.

(2) For example, D. H. R. Barton, D. A. Lewis, and J. F. McGhie, *J. Chem. Soc.*, 2907 (1957); C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill Book Co., Inc., New York, N. Y., 1960, p. 126.

(3) N. Allinger and M. A. DaRooge, *Tetrahedron Letters*, **19**, 676 (1961); *J. Am. Chem. Soc.*, **84**, 4561 (1962).

(4) J. Lehn, J. Levisalles, and G. Ourisson, *Tetrahedron Letters*, **19**, 682 (1961).

(5) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).

(6) K. L. Williamson and W. S. Johnson, *J. Am. Chem. Soc.*, **83**, 4623 (1961).

(7) J. Y. Chien, *J. Chem. Educ.*, **24**, 494 (1947).

(8) I. F. Halverstadt and W. D. Kumler, *J. Am. Chem. Soc.*, **64**, 2988 (1942).

TABLE I

DIPOLE MOMENT DATA

ω_2	ϵ_{12}	ν_{12}		
Cholestane (I)				
0.01694966	2.2749	1.14390	α = 0.060	ϵ_1 = 2.2738
.01014860	2.2745	1.14430	β = −0.060	ν_1 = 1.14489
.00845719	2.2742	1.14430	$P_{2\infty}$ = 124.7 cc.	R_D = 118.1 cc.
.00428205	2.2740	1.14469	μ = 0.22 D.	
Cholestan-3-one (II)				
0.01560673	2.3167	1.14130	α = 2.771	ϵ_1 = 2.2739
.00850687	2.2975	1.14325	β = −0.244	ν_1 = 1.14516
.00732450	2.2945	1.14338	$P_{2\infty}$ = 305.4 cc.	R_D = 130.0 cc.
.00370442	2.2841	1.14417	μ = 3.03 D.	
2α-Bromocholestan-3-one[9] (III)				
0.01744167	2.3486	1.14026	α = 4.348	ϵ_1 = 2.2731
.00764927	2.3060	1.14325	β = −0.316	ν_1 = 1.14576
.00530761	2.2970	1.14417	$P_{2\infty}$ = 496.1 cc.	R_D = 125.9 cc.
.00374177	2.2880	1.14456	μ = 4.27 D.	
2α,4α-Dibromocholestan-3-one[10] (IV)				
0.01329605	2.3384	1.14091	α = 4.683	ϵ_1 = 2.2760
.00816799	2.3152	1.14208	β = −0.351	ν_1 = 1.14527
.00589400	2.3031	1.14260	$P_{2\infty}$ = 608.2 cc.	R_D = 133.7 cc.
.00342010	2.2920	1.14690	μ = 4.83 D.	
2ζ-Chloro,2ζ-bromocholestan-3-one[11] (V)				
0.01842472	2.3495	1.13806	α = 4.080	ϵ_1 = 2.2743
.00674857	2.3027	1.14182	β = −0.349	ν_1 = 1.14442
.00649568	2.2994	1.14208	$P_{2\infty}$ = 501.9 cc.	R_D = 133.7 cc.
.00414537	2.2916	1.14325	μ = 4.26 D.	
3-Desoxyallobetulone (VI)				
0.01351893	2.2842	1.14156	α = 0.738	ϵ_1 = 2.2741
.00671145	2.2784	1.14312	β = −0.215	ν_1 = 1.14449
.00476471	2.2779	1.14338	$P_{2\infty}$ = 177.4 cc.	R_D = 129.2 cc.
.00273914	2.2762	1.14391	μ = 1.54 D.	
Allobetulone (VII)				
0.01726696	2.3140	1.14092	α = 2.278	ϵ_1 = 2.2751
.01284445	2.3052	1.14221	β = −0.201	ν_1 = 1.4452
.00541322	2.2867	1.14299	$P_{2\infty}$ = 312.6 cc.	R_D = 129.2 cc.
.00385866	2.2835	1.14404	μ = 3.00 D.	
2α-Bromoallobetulone (VIII)				
0.01176193	2.3102	1.14117	α = 3.133	ϵ_1 = 2.2732
.00753980	2.2980	1.14299	β = −0.272	ν_1 = 1.14462
.00414318	2.2843	1.14352	$P_{2\infty}$ = 441.3 cc.	R_D = 137.0 cc.
.00146236	2.2788	1.14404	μ = 3.87 D.	
2β-Bromoallobetulone (IX)				
0.00959909	2.3220	1.14208	α = 4.877	ϵ_1 = 2.2744
.00724059	2.3094	1.14247	β = −0.353	ν_1 = 1.14531
.00536355	2.3002	1.14352	$P_{2\infty}$ = 599.3 cc.	R_D = 137.0 cc.
.00185458	2.2835	1.14459	μ = 4.77 D.	
2α,2β-Dibromoallobetulone (X)				
0.01345447	2.3279	1.13845	α = 4.009	ϵ_1 = 2.2741
.00745591	2.3052	1.14052	β = −0.459	ν_1 = 1.14442
.00685524	2.3005	1.14104	$P_{2\infty}$ = 573.2 cc.	R_D = 144.8 cc.
.00480183	2.2933	1.14273	μ = 4.59 D.	

Theoretical Calculations

Dipole Moments.—Table II shows theoretical dipole moments calculated for only five of the many possible conformations of ring A; namely, chair, planar 1, boat 1, planar 2, and boat 2 (see Fig. 1, 2, 3, 4, and 5). In planar 1, C_1, C_2, C_3, and C_4 lie in a common plane; in planar 2, C_1, C_2, C_3, and C_{10} lie in a common plane; in boat 1, C_3 and C_{10} represent the apexes; in boat 2, C_2 and C_5 represent the apexes. These calculations are based on the reasonable assumption that the tetravalent carbon has tetrahedral valence bonds and trivalent carbon has trigonal valence bonds. Furthermore, the C–C bond length is assumed to be constant throughout the ring. The fact that cholestane has a very small dipole moment, 0.22 D., is evidence that the dipoles of its derivatives can be attributed almost entirely to the bond moments of polar substituents. Vector addition of bond moments was carried out by a method similar to that described by Brutcher and Bauer[12] for substituted cyclopentanes. The coordinate axes were chosen, as shown in Fig. 1, 2, 3, 4, and 5, so that C_6, C_8, C_{10}, C_{11}, and C_{13} lie in the x,y-plane which coincides with the over-all plane of the molecule. Unit

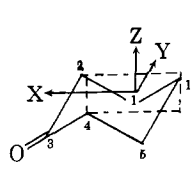


Fig. 1.—Chair.

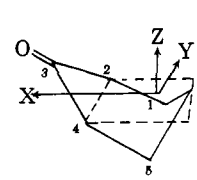


Fig. 2.—Planar 1.

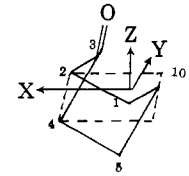


Fig. 3.—Boat 1.

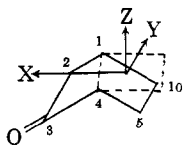


Fig. 4.—Planar 2.

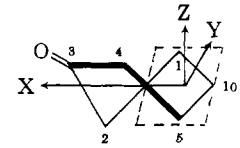


Fig. 5.—Boat 2.

vectors locating the x,y,z-coordinates of various substituents for the various forms of ring A are listed in Table III. Utilization of the unit vectors provides a convenient way of calculating dipole moments for the various conformations. The absolute value of the over-all dipole moment of a molecule is given by the square root of the dot product

$$|\mu| = (\vec{\nu} \cdot \vec{\nu})^{1/2}$$

where

$$\vec{\nu} = \sum_i u_i \vec{\nu}_i$$

in which u_i is the bond moment and $\vec{\nu}_i$ is the unit vector describing the coordinates of polar substituent i.

TABLE II

OBSERVED AND CALCULATED DIPOLE MOMENTS (DEBYE UNITS)

Compound	μ (exptl.)	μ (calcd.) Chair	Planar 1	Boat 1	Planar 2	Boat 2	Apparent conformation
Cholestane	0.22	..	..	..	..	..	...
Cholestan-3-one	3.03	3.03	3.03	3.03	3.03	3.03	Chair
2α-Br-	4.27	4.23	3.91	2.33	3.91	2.33	Predom. chair
2α-Br,4α-Br-	4.83	4.86	5.01	3.14	4.89	3.80	Predom. chair
2-Cl,2-Br-	4.26	3.40	4.25	3.40	4.25	3.40	Planar
3-Desoxyallobetulone	1.54	1.54	1.54	1.54	1.54	1.54	...
Allobetulone	3.00	2.54	3.81	4.48	3.17	3.74	Distort. chair
2α-Br-	3.87	3.88	4.20	3.39	3.36	2.18	Distort. chair
2β-Br-	4.77	2.93	5.12	5.73	4.55	4.71	Planar or boat
2α-Br,2β-Br-	4.59	3.78	5.05	4.63	4.29	3.22	Planar or boat
Cyanolupan-3-one	3.93[a]	3.18	5.54	6.70	4.34	5.24	Distort. chair

[a] Taken from reference 4.

In the theoretical calculations we have employed the following bond moments: C=O, 3.03 D. from cholestan-3-one; ether oxygen in allobetulone, 1.54 D. from 3-desoxyallobetulone; C—Br and C—Cl, 1.90 D. Furthermore we have assumed the following inductive effects based on dipole moments of a series of simple substituted cyclohexanones[13,14]: an α-halogen lowers a C=O bond moment to 2.84 D.; two α-halogens lower a C=O bond moment to 2.67 D.; two halogens on the same carbon reduce the carbon–halogen bond moment to 1.82 D.

(9) L. F. Fieser and X. A. Dominguez, *J. Am. Chem. Soc.*, **75**, 1704 (1953).

(10) C. Djerassi and C. R. Scholz, *ibid.*, **69**, 2404 (1947).

(11) J. J. Beereboom and C. Djerassi, *J. Org. Chem.*, **19**, 1196 (1954).

(12) F. V. Brutcher, Jr., and W. Bauer, Jr., *J. Am. Chem. Soc.*, **84**, 2233 (1962).

(13) W. K. Kumler and A. C. Huitric, *ibid.*, **78**, 3369 (1956).

(14) P. Bender, D. L. Flowers, and H. L. Goering, *ibid.*, **77**, 3463 (1955).

TABLE III

UNIT VECTORS FOR VARIOUS SUBSTITUENTS FOR CHAIR, PLANAR, AND BOAT CONFORMATIONS OF RING A

Position	Group	x	y	z
		Chair		
3	Keto	0.81647	0.00000	−0.57738
2	α-Halogen	.47142	.81653	−0.33326
2	β-Halogen	.00000	.00000	1.00000
4	α-Halogen	.47142	− .81653	−0.33326
	Ether oxygen[a]	.00000	.30902	0.95106
		Planar 1		
3	Keto	0.94284	0.00000	0.33326
2	α-Halogen	.58641	.47141	− .65870
2	β-Halogen	.04222	.47141	.88090
4	α-Halogen	.58641	− .47141	− .65870
	Ether oxygen[a]	.00000	.30902	.95106
		Boat 1		
3	Keto	0.27228	0.00000	0.96222
2	α-Halogen	.62842	.00000	− .77788
2	β-Halogen	.15720	.81647	.55557
4	α-Halogen	.62842	.00000	− .77788
	Ether oxygen[a]	.00000	.30902	.95106
		Planar 2		
3	Keto	0.97928	0.06322	−0.19242
2	α-Halogen	.13614	.23580	− .96222
2	β-Halogen	.40824	.70709	.57738
4	α-Halogen	.47142	− .81652	− .33326
	Ether oxygen[a]	.00000	.30902	.95106
		Boat 2		
3	Keto	0.95257	0.23568	0.19253
2	α-Halogen	− .31420	− .54422	− .77788
2	β-Halogen	.47142	.81652	− .33326
4	α-Halogen	.47142	− .81652	− .33326
	Ether oxygen[a]	.00000	.30902	.95106

[a] Ether oxygen in allobetulones. The unit vector was determined from Dreiding models.

N.m.r. Spectra.—From theoretical considerations Karplus[5] has shown that the spin–spin coupling of protons bonded to adjacent carbon atoms is a sensitive function of the dihedral angle, ϕ, between the carbon–hydrogen bonds. Williamson and Johnson[6] in an attempt to apply the Karplus equation to conformational studies of some acetoxycholestanones arrived at the revised equation

$$J_{HH'} = \begin{cases} 10\cos^2\phi & 0° \leq \phi \leq 90° \\ 16\cos^2\phi & 90° \leq \phi \leq 180° \end{cases}$$

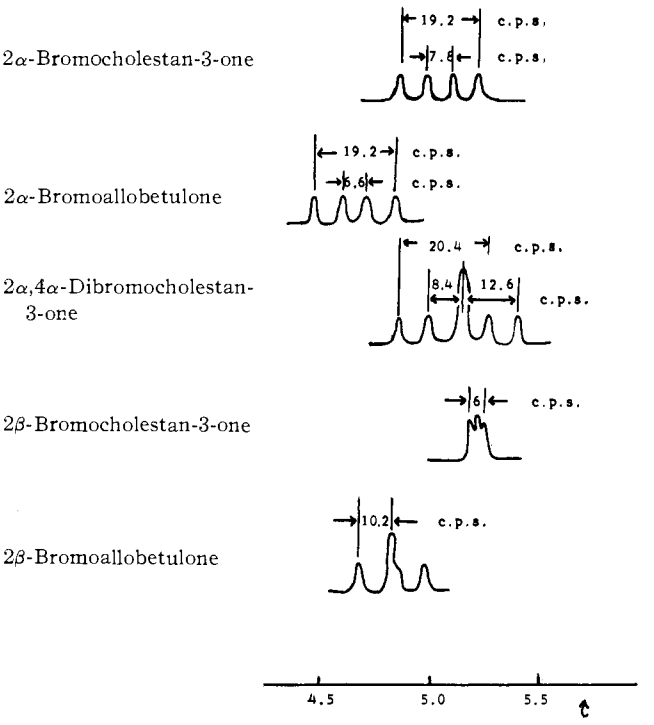
Since the compounds under study here are very similar to the acetoxycholestanones, one should be able to apply this modified equation to the present problem. However, with the present state of knowledge, one has to accept the deductions from the Karplus equation with some reservation.

The n.m.r. spectra of the protons bonded to the same carbon as the bromine atom are illustrated in Table IV. In the case of 2α-bromocholestan-3-one, for example, the signal of this proton is split by the two protons on C_1. The resulting four-line pattern is typical of an X proton in an ABX system.[15] Although the distance between the two outer lines is equal to $J_{AX} + J_{BX}$, the distance between the two inner lines is a complex function of the *gem* coupling constant, J_{AB}, and the chemical shift, δ_{AB}, between protons A and B, namely

$$|\{[\delta_{AB} + {}^1/_2(J_{AX} - J_{BX})]^2 + J_{AB}^2\}^{1/2} - \{[\delta_{AB} - {}^1/_2(J_{AX} - J_{BX})]^2 + J_{AB}^2\}^{1/2}|$$

(15) J. A. Pople, W. G. Schneider, and H. J. Bernstein, "High Resolution Nuclear Magnetic Resonance," McGraw-Hill Book Co., Inc., New York, N. Y., 1959.

TABLE IV



If δ_{AB} is much greater than J_{AB} the distance between the two inner lines is $J_{AX} - J_{BX}$ and one can then obtain the coupling constants. Since the signals of the two *gem* protons A and B are lost in the mass of signals upfield, it is not possible to determine δ_{AB} and, consequently, one cannot solve for the coupling constants without invoking some sort of assumption. The *gem* proton–proton coupling constant has been shown[16] to be approximately 12 c.p.s. for tetrahedral bonds, which is the situation here. Because the *gem* protons have similar environments the chemical shift between these two protons is expected to be small, probably not much greater than 12 c.p.s. Unfortunately, for this reason we cannot determine the coupling constants without risking serious errors.

When the distance between the two inner lines equals zero, as in 2β-bromocholestan-3-one[17] and almost zero in 2β-bromoallobetulone, then J_{AX} equals J_{BX}. Assignments of 3.0 and 10.2 c.p.s. for the proton–proton couplings are made for these two molecules, respectively.

In determining the coupling constant between the 4α-hydrogen and 5β-hydrogen in 2α,4α-dibromocholestan-3-one no such complication arises. The 4α-hydrogen is split into a doublet indicating a coupling constant of 12.6 c.p.s. (see Table IV).

Discussion

On the basis of conformational analysis the predominant conformation of ring A in steroids and analogous compounds is a chair. The boat conformation has been suggested when strong nonbonded interactions are present, for example in some substituted 4,4-dimethyl-steroids.[2] Although the chair form is unique for the A ring there are two extreme boat forms: boat 1 (see Fig. 3) in which C_3 and C_{10} represent the apexes, and boat 2 (see Fig. 5) in which C_2 and C_5 represent the apexes. Allinger and DaRooge[3] have drawn special attention to a planar form (planar

(16) H. S. Gutowsky, M. Karplus, and D. M. Grant, *J. Chem. Phys.*, **31**, 1278 (1959).

(17) Prepared by the method of G. H. Alt and D. H. R. Barton, *J. Chem. Soc.*, 4284 (1954).

1, see Fig. 2) which is intermediate between the chair and boat 1 forms. However, there is another planar form (planar 2, see Fig. 4) which is intermediate between the chair and boat 2 forms. Numerous other conformations are, of course, possible for ring A, but for the purpose of simplicity we will restrict our discussion to the chair, boat, and planar forms.

Although the calculations here are based on static models no implications are intended regarding the possibility of dynamic equilibrium. The measured dipole moment is, of course, the net resultant of moments corresponding to the various conformations that the molecule assumes under given conditions of temperature and solvent.

It is safe to assume that cholestan-3-one is in the true chair form. In the case of allobetulone, however, which has the 4,4-dimethyl group, our calculations (see Table II) clearly indicate that the A ring can neither be a true chair nor one of the boat forms. The experimental moment best corresponds to a form intermediate between a chair and the two planar forms.

Lehn, Levisalles, and Ourisson[4] have studied a similar molecule containing a cyano group, the moment of which was used as a probe in determining the shape of ring A. We have recalculated the theoretical moments for cyanolupan-3-one (see Table II) using our system of coordinates. The conclusions about the shape of ring A in cyanolupanone are exactly analogous to those in allobetulone, for which we have used the ether moment as a probe. It is satisfying to note that measurements on two different molecules incorporating two different probes lead to the same conclusions regarding the stereochemistry of ring A.

Allinger and DaRooge[3] have studied 4,4-dimethyl-3-keto-steroids (II, A = B = CH_3) which differ from the two systems mentioned above by the absence of the 8-methyl group. Using the 17-keto moment as a probe they have also observed for ring A a departure from the chair form.

Stereochemistry of 2α-Haloketones.—We have extended our studies to α-haloketones. From dipole moments (see Table II), we find that the A ring in 2α-bromocholestan-3-one and 2α-bromoallobetulone is essentially a chair: these molecules are similar to their respective parent ketones.

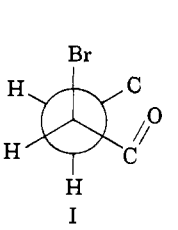
Further information concerning the stereochemistry of these bromoketones is given by their n.m.r. spectra. A proton on a carbon carrying a halogen appears in the 5 τ region and is therefore distinct from most other protons. The signals of the 2β-protons in the bromo ketones under study are split by the adjacent protons on C_1. The resulting pattern has been shown by Karplus[5] to be a sensitive function of the dihedral angles between the adjacent protons. The observation that the n.m.r. pattern for the 2β-H of 2α-bromocholestan-3-one and 2α-bromoallobetulone are extremely similar (see Table IV) indicates that the conformation of the A ring in these two molecules must be very similar. It is interesting to note that the pattern for the 2β-hydrogen in 2α-iodo-, 2α-fluoro-, and 2α-acetoxy[6]-cholestan-3-ones are very similar in spite of the large variation in the size and electronegativity of the 2α-substituent.

Thus on the basis of dipole moment and n.m.r. spectra we conclude that these molecules all have a very similar conformation, namely, a slightly distorted chair form for the A ring. This is to be expected on the basis of conformational analysis because the substituents are in the equatorial position and therefore are not subject to strong nonbonded interaction.

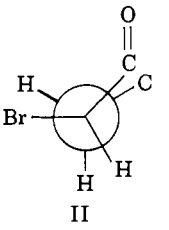
Stereochemistry of 2α,4α-Dihaloketone.—The dipole moment of 2α,4α-dibromocholestan-3-one (see Table II) clearly excludes the boat conformations for ring A. Since the calculated dipole moments for the chair and planar forms are close to one another and also to the experimental value, a definite assignment on the basis of dipole moment cannot be made. The n.m.r. spectra in the 5-τ region can be resolved into two groups, a four-line pattern typical for the 2β-proton and a two-line pattern for the 4β-proton. The four-line pattern is almost identical with that observed for the 2α-haloketone described above. In the light of the Karplus[5] equation and the work of Williamson and Johnson,[6] the coupling constant, 12.6 c.p.s., for the 4β-hydrogen indicates that the dihedral angle between the 4β- and 5α-protons is close to 180°, *i.e.*, the 4β- and 5α-hydrogens are nearly diaxial. This approximates a chair conformation.

Stereochemistry of 2β-Haloketones.—The dipole moment of 2ζ-chloro-2ζ-bromocholestan-3-one (see Table II) clearly excludes the chair and boat forms, but strongly favors the planar forms. In the corresponding compound in the other series, 2α,2β-dibromoallobetulone, the dipole moment also rules out the chair and boat 2 forms. It seems likely that the A ring is intermediate between boat 1 and the planar forms. In the case of 2β-bromoallobetulone the chair and boat 1 forms are ruled out, but a conformation intermediate between boat 2 and the planar forms is favored.

It was not possible to measure the dipole moment of 2β-bromocholestan-3-one because of its instability. Its n.m.r. spectrum, however, could be recorded. The pattern for the 2α-proton consisted of three lines (see Table IV) for which one could assign two equal coupling constants of approximately 3.0 c.p.s. These constants correspond to the couplings between the 2α-H and the two protons on C_1. Using the modified Karplus equation of Williamson and Johnson[6] (*vide supra*), one finds that these numbers correspond to the 2α-proton occupying an almost gauche arrangement as shown in Newman projection I. This arrangement corresponds approximately to a chair conformation



I



II

The spectra of the corresponding proton in 2β-fluoro-5α-androstan-3,17-dione, taken in the same solvent, is very similar.[18] Furthermore, the dipole moment of the fluoro compound has been interpteted as indicative of a departure from the chair conformation for ring A.

In the case of 2β-bromoallobetulone the n.m.r. spectrum of the 2α-proton is again an almost three-line pattern with nearly equal coupling constants of 10.2 c.p.s. From the Karplus equation one is led to a conformation approximated by Newman projection II, which corresponds to planar 2 or boat 1.

Conclusions.—On the basis of conformational analysis a 2β-halogen will have substantial interaction with the 19-methyl group, which in turn will lead to some deformation of the chair form of ring A. The addition of a 4β-methyl group will considerably increase the nonbonded interaction between these groups above the plane of the ring and will lead to an even further deformation of ring A. In the case of 2α,2β-dibromoallobetulone the important nonbonded interactions for the

(18) N. L. Allinger, M. A. DaRooge, M. A. Miller, and B. Waegell, private communication.

chair and boat forms of ring A are listed in Table V. These nonbonded interactions are considerably decreased in the planar forms. Planar 1 is more favorable than planar 2 since a stronger interaction between the 19-methyl and 4β-methyl groups is present in the latter case.

TABLE V

Conformation		Important nonbonded interactions
Chair	C	2β-Br:19-Me:4β-Me
Boat 1	B-1	CO:19-Me:2α-Br:4α-Me
Boat 2	B-2	19-Me:4β-Me:2α-Br:5α-H

We thus find that the conclusions based on dipole moment data and n.m.r. spectra are in substantial agreement with the results of conformational analysis. Each of these methods has important limitations and the results derived from a consideration of only one of these methods could be unreliable. Bond angle distortions of a few degrees can occur at different points throughout the molecule, and consequently one cannot expect dipole moment or other physical measurements to correspond exactly to the calculated value. In the cases studied here, however, all of these methods essentially pointed to the same stereochemistry. These findings can therefore be accepted with confidence as a first approximation to the real state of affairs.

Acknowledgment.—We are thankful to Professor A. Vystrčil and Dr. J. Klinot for samples of allobetulone derivatives and to Professor G. Ourisson,[19] Dr. J. M. Lehn, and Dr. N. L. Allinger for valuable discussions. The computations were programmed by Dr. P. Funke and carried out at the Computer Center[20] of Stevens Institute of Technology, for which we record our appreciation. This work was supported in part by a grant (G-13290) from the National Science Foundation and a grant (CA-05079) from the Cancer Institute of the U. S. Public Health Service.

(19) In a paper presented before the Second International Symposium on the Chemistry of Natural Products, Prague, 1962, J. M. Lehn, J. Levisalles, G. Ourisson, and P. Witz described their studies on triterpenes which are parallel to ours.

(20) Supported in part by a grant from the National Science Foundation.

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Cleavage of Histidyl Peptide Bonds by N-Bromosuccinimide[1]

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RECEIVED APRIL 23, 1963

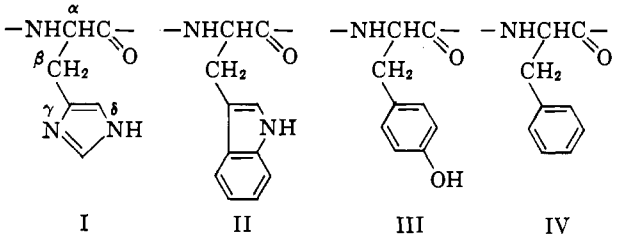
A nonenzymatic method for cleavage of C-histidyl peptide bonds was developed. The imidazole ring of histidine residues is oxidized by N-bromosuccinimide (NBS) in pyridine acetate buffers (pH 3–4) at room temperature. After destruction of excess NBS the reaction mixture is heated for 1 hr. at 100°. Yields of cleavage were found to be 50–65% in histidyl dipeptide models and 30–55% in several synthetic polypeptides consisting of 5–10 amino acids. The histidyl-prolyl bond in sperm-whale myoglobin was cleaved in 53% yield. Tryptophyl and tyrosyl peptide bonds are also cleaved by NBS. By adjustment of reaction conditions and the use of masking groups, differentiation of cleavage of peptide bonds next to tryptophan, tyrosine, and histidine was achieved.

Introduction

In the last few years a new approach to sequence analysis and fragmentation of proteins has been developed. The specific chemical reactivity of certain amino acid residues, permits cleavage of peptide bonds at these sites by nonenzymatic reagents. In many cases, the amino acid residue is selectively modified prior to cleavage. This modification in itself is of potential value in studying the correlation between structure and biological function of proteins. Several nonenzymatic methods for selective cleavage of peptide bonds are reported in the literature. Some of these methods were successfully applied to polypeptides and proteins.[3-14] The work done in this field was recently reviewed by Witkop.[15]

We wish to report here an oxidative method for cleavage of histidyl peptide bonds. No proteolytic enzyme is known to cleave these bonds selectively. Furthermore, histidyl residues are thought to be involved in the biological activity of several enzymes.[16] Therefore, a selective method of cleavage may serve as a tool for the determination of sequence near the active sites of these proteins.

Histidyl residues (I) have a double bond in the γ–δ position relative to the carbonyl group of the peptide bond. A similarly located double bond is found in tryptophyl (II), tyrosyl (III), and phenylalanyl (IV) residues.



I II III IV

When brominating reagents, such as N-bromosuccinimide (NBS), N-bromoacetamide, or bromine, react with tryptophyl or tyrosyl residues, cleavage of their C–peptide bond occurs.[3,17,18] The mechanism sug-

(1) Presented in part in the 1961 and 1963 Meetings of the Israel Chemical Society (S. Shaltiel and A. Patchornik, *Bull. Res. Council Israel*, **10A**, 48 (1961), and "Proceedings of the XXXII Meeting of the Israel Chem. Soc.," 1963, p. 19).

(2) Part of a Ph.D. thesis to be submitted to the Hebrew University, Jerusalem.

(3) A. Patchornik, W. B. Lawson, E. Gross, and B. Witkop, *J. Am. Chem. Soc.*, **82**, 5923 (1960).

(4) L. K. Ramachandran and B. Witkop, *ibid.*, **81**, 4028 (1959).

(5) E. Gross and B. Witkop, *J. Biol. Chem.*, **237**, 1856 (1962).

(6) A. Patchornik and M. Sokolovsky, "Proceedings of the Vth European Peptide Symposium," 1962, in press.

(7) S. Sarid and A. Patchornik, "Proceedings of the XXXII Meeting of the Israel Chem. Soc.," 1963, p. 18.

(8) F. A. Quiocho, M. O'Dell, and F. Friedberg, *Experientia*, **17**, 217 (1961).

(9) T. Peters, *Compt. rend. trav. lab. Carlsberg*, **31**, 227 (1959).

(10) L. K. Ramachandran, *Biochim. Biophys. Acta*, **41**, 524 (1960); *J. Sci. and Ind. Research*, **21c**, 111 (1962).

(11) I. Bernier and P. Jolles, *Compt. rend. acad. sci., Paris*, **253**, 745 (1961).

(12) G. F. Grannis, *Arch. Biochem. Biophys.*, **91**, 255 (1960).

(13) J. Schultz, H. Allison, and M. Grice, *Biochem.*, **1**, 694 (1962).

(14) D. T. Gish, *Biochim. Biophys. Acta*, **35**, 557 (1959); *J. Am. Chem. Soc.*, **82**, 6329 (1960).

(15) B. Witkop in "Advances in Protein Chemistry," Vol. 16, C. B. Anfinsen, M. L. Anson, K. Bailey, and J. T. Edsall, Ed., Academic Press, Inc., New York, N. Y., 1961, pp. 221–321.

(16) E. A. Barnard and W. D. Stein in "Advances in Enzymology," Vol. XX, F. F. Nord, Ed., Interscience Publishers, Inc., New York, N. Y., 1958, pp. 51–110.

(17) G. Schmir, L. Cohen, and B. Witkop, *J. Am. Chem. Soc.*, **81**, 2228 (1959).

(18) E. J. Corey and L. F. Haefele, *ibid.*, **81**, 2225 (1959).