Further studies of the reactivity of chlorocarbene and the different behavior of methylene bromide toward butyllithium will be the subject of a detailed publication.

DEPARTMENT OF CHEMISTRY
THE UNIVERSITY OF CHICAGO
CHICAGO 37, ILL.
GERHARD L. CLOSS
LISELOTTE E. CLOSS

RECEIVED JULY 9, 1959

CHROMATOGRAPHY OF MYOSIN

Sir:

The general method of Peterson and Sober[1] has been applied to the muscle protein, myosin or "myosin A." Myosin A[2,3,4] freed of myosin B by dialysis against 0.2 *M* KCl, 0.01 *M* tris *p*H 7.4 in the presence of adenosine triphosphate and by 1 hour of centrifugation at 55,000 × *g* was passed through a diethylaminoethyl cellulose column equilibrated with a solvent 0.2 *M* KCl, 0.01 *M* tris *p*H 7.4. An ascending gradient to 1.0 *M* KCl was applied (Fig. 1), and protein concentration was measured[5] in the effluent. Protein recovery was better than 80%.

TABLE I

Prepn.		α	β
19	$\bar{M}_w \times 10^{-5}$	4.52	6.10
	$\bar{r}_g$	437	474
	V_m (2 d.)	4.7	9.5
22	$\bar{M}_w \times 10^{-5}$	4.55	5.00
	$\bar{r}_g$	434	560
	V_m (12 d.)	0.4	3.8
28	$\bar{M}_w \times 10^{-5}$	4.02	5.60
	$\bar{r}_g$	475	634
	V_m (3 d.)	5.0	17
33	$\bar{M}_w \times 10^{-5}$	4.21	6.36
	$\bar{r}_g$	430	500
	V_m (0 d.)	8.0	8.7
	V_m (11 d.)	1.0	8.0
21	$\bar{M}_w \times 10^{-5}$	4.00	..
	$\bar{r}_g$	434	..

Myosin is resolved into at least two components, α and β (Fig. 1). Neither component shows a turbidity drop on adenosine triphosphate addition, confirming the elimination of myosin B. The α-component probably is highly purified myosin. The data[6] of Table I yield an average $\bar{M}_w$ of 4.3 × 10^5 g. and an average $\bar{r}_g$ of 442 Å. $\bar{M}_w$ from ultracentrifuge work[7] is 4.2 × 10^5 g. This shows that the two methods can agree; moreover the straightness of the Zimm light-scattering plot (Fig. 1) does not encourage speculation about myosin nonuniform substructure.[8] In this work the "full" Zimm plot (*i.e.*, intensities at various concentrations as well as at various angles) was not attempted because it has been shown[8] that in 0.6 *M* KCl the second virial coefficient is essentially zero. The β-component is heavier (average $\bar{M}_w$, 5.77 × 10^5 g.) and more extended (average $\bar{r}_g$ 542 Å.); also its specific ATPase activity,[9] V_m, (Table I) is greater and more thermostable than that of the α-component. Scattered observations suggest that β may be transformable into α, either by warming briefly from 4 to 25°, or by aging.

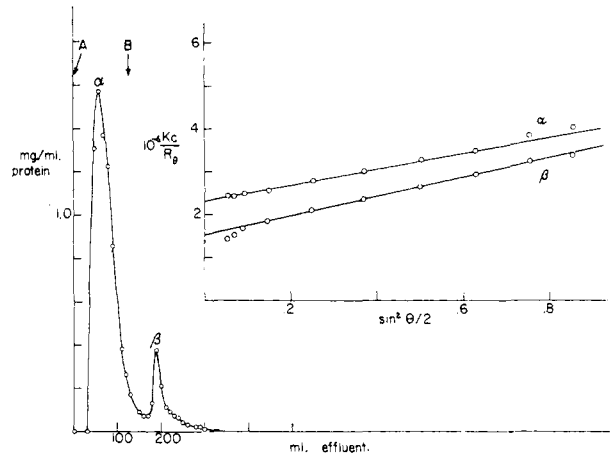


Fig. 1.—Chromatography on a 13 × 2.5 cm. column of diethylaminoethyl cellulose (1 meq./g.); eluting solutions: A—0.2 *M* KCl, 0.01 *M* tris *p*H 7.4; B—gradient elution to 1.0 *M* KCl; flow rate 60 ml./hr.; 10 ml. fractions were collected. The gradient used was composed of two cone-cylindrical vessels filled with 250 ml. of 1.0 *M* KCl, 0.01 *M* tris *p*H 7.4 and 125 ml. of 0.2 *M* KCl, 0.01 *M* tris *p*H 7.4. Insert shows: Zimm plot of α and β fractions in 0.5 *M* KCl, 0.01 *M* tris *p*H 7.4.

The author is indebted to Dr. M. Gellert for guidance in light-scattering measurements, to Dr. M. F. Morales for general counsel, and to Dr. W. Niemierko for valuable criticisms. This work was supported by a Rockefeller Fellowship and by Training Grant 2G-174 of the U.S.P.H.S.

(1) E. Peterson and H. A. Sober, THIS JOURNAL, **78**, 751 (1956).

(2) A. Szent-Györgyi, "Muscular Contraction," Academic Press, Inc., New York, N. Y., 1947.

(3) Dr. J. Botts, private communication.

(4) H. H. Weber and H. Portzehl, *Advances in Protein Chemistry*, **7**, 161 (1952).

(5) O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

(6) For specific refractive index increment the value of 0.209 ml./g. was used.

(7) P. H. von Hippel, H. K. Schachman, P. Appel and M. F. Morales, *Biochim. Biophys. Acta*, **28**, 504 (1958).

(8) A. Holtzer and S. Lowey, THIS JOURNAL, **81**, 1370 (1959).

(9) μ mole P·sec.$^{-1}$ g. protein^{-1} in 0.5 *M* KCl, 0.1 *M* tris, 10^{-3} $CaCl_2$, *p*H 8.0, 25°. The age of myosin preparation (in days) is indicated in parentheses.

DEPARTMENT OF BIOCHEMISTRY
DARTMOUTH MEDICAL SCHOOL
HANOVER, N. H.
J. BRAHMS

RECEIVED JULY 6, 1959

OPTICAL ROTATORY DISPERSION STUDIES. XXX.[1] DEMONSTRATION OF BOAT FORM IN A 3-KETO STEROID[2]

Sir:

Kinetically controlled bromination of 2α-methylcholestan-3-one[3] (or of its enol acetate) leads to 2-bromo-2-methylcholestan-3-one (m.p. 136–138°), whose spectral properties ($\lambda_{max}^{CHCl_3}$ 5.84 μ; $\lambda_{max}^{cyclohex}$ 313 mμ) require[4] an axial bromine atom. By

(1) Paper XXIX, P. Crabbé, C. Djerassi, E. J. Eisenbraun and S. Liu, *Proc. Chem. Soc.*, in press.

(2) Supported by grant No. CY-2919 from the National Cancer Institute.

(3) Y. Mazur and F. Sondheimer, THIS JOURNAL, **80**, 5220 (1958).

(4) (a) R. N. Jones, D. A. Ramsay, F. Herling and K. Dobriner, *ibid.*, **74**, 2828 (1952); (b) R. C. Cookson, *J. Chem. Soc.*, 282 (1954).

reasonable analogy to earlier generalizations,[5] the bromo ketone was assigned[3] the expected 2β-bromo-2α-methylcholestan-3-one (I) formulation. We now wish to demonstrate that the situation is considerably more complicated as was uncovered by means of optical rotatory dispersion.[6]

According to the "axial haloketone rule,"[7,8] a 2β-bromo-2α-methyl-3-keto steroid in the chair form (IA) should exhibit a positive Cotton effect, contrary to the observed (Fig. 1) negative one of

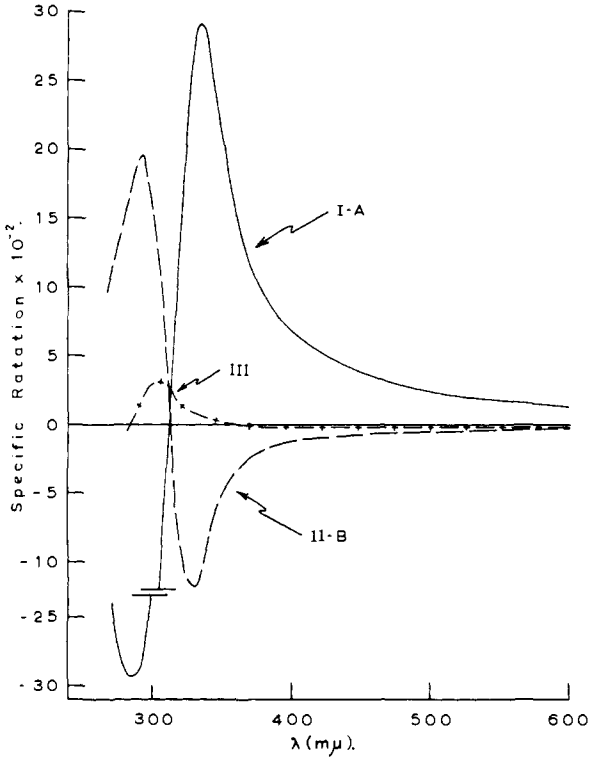
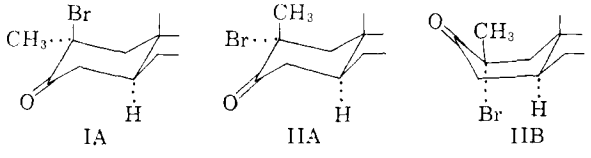


Fig. 1.

the bromo ketone (m.p. 136–138°).[3] The boat form IIB of the 2α-bromo-2β-methyl-3-keto isomer, however, satisfies all criteria, the bromine atom occupying an axial orientation and the "axial haloketone rule"[7,8] predicting a negative Cotton effect.



Conclusive support for this supposition is afforded by hydrogen bromide–acetic acid equilibration of 2α-bromo-2β-methylcholestan-3-one (II) (m.p. 136–138°) which yielded 2β-bromo-2α-methylcholestan-3-one (I) (m.p. 120–122°, $\lambda_{max}^{dioxane}$ 308 mμ, $\lambda_{max}^{CHCl_3}$ 5.85 μ) and 2α-methyl-4α-bromocholestan-3-one (III) ($^{max}_{m.p.}$ 140–141°, $\lambda_{max}^{CHCl_3}$ 5.77 μ, $\lambda_{inflect.}^{dioxane}$ 285 mμ). The position of the bromine atom was established by dehydrobromination, I yielding Δ¹-2-methylcholesten-3-one[8] and III giving 2α-methyl-Δ⁴-cholesten-3-one.[8,9] The spectral properties of I demonstrate that the bromine atom is axial and its powerful, positive Cotton effect curve (Fig. 1) is fully consistent[7,8] with the chair formulation IA. Similarly, the spectral and rotatory dispersion (Fig. 1) data show that the bromine atom in III is equatorially oriented. Identical isomers (I, II, III) were encountered in the 2α-methylandrostan-17β-ol-3-one series.

The above results have several important implications: (a) the "axial haloketone rule"[7] is applicable to boat as well as chair forms; (b) chair form IA is energetically favored over the boat IIB, but the latter is preferred over its corresponding chair form IIA. That this is due to the presence of the angular methyl group (the chair form IIA having the electrostatically unfavorable equatorial orientation of the bromine as well as a 1,3-diaxial dimethyl interaction, none of which are found in the boat IIB) will be demonstrated in a forthcoming paper reporting the bromination of analogous 19-nor steroids and where no boat form is encountered.

The fact that the product of the kinetically controlled bromination is II, while the thermodynamic product is I appears to be contrary to the earlier generalizations.[5] We intend to comment on this point in another paper, together with additional experimental evidence.

(5) E. J. Corey, THIS JOURNAL, **76**, 175 (1954).
(6) See C. Djerassi, *Bull. Soc. Chim. France*, 741 (1957).
(7) C. Djerassi and W. Klyne, THIS JOURNAL, **79**, 1506 (1957).
(8) C. Djerassi, J. Osiecki, R. Riniker and B. Riniker, *ibid.*, **80**, 1216 (1958).
(9) J. A. K. Quartey, *J. Chem. Soc.*, 1710 (1958).
(10) Stanford University, Stanford, California.

DEPARTMENT OF CHEMISTRY
WAYNE STATE UNIVERSITY
DETROIT, MICHIGAN, AND
SYNTEX, S. A.
APDO. POSTAL 2679
MEXICO, D. F.

CARL DJERASSI[10]
NEVILLE FINCH
ROLF MAULI

RECEIVED JULY 15, 1959

DEUTERIUM EXCHANGE OF DECABORANE WITH DEUTERIUM CHLORIDE UNDER ELECTROPHILIC CONDITIONS

Sir:

In contrast to the recently reported base catalyzed deuterium exchange reactions of decaborane[1,2] with such compounds as deuterium oxide and chloride, we wish to report the results of an electrophilic exchange study.

Four successive exchanges of a 10 mmole sample of decaborane with 130 mmole portions of deuterium chloride in the presence of 10 mmole of aluminum chloride and in carbon disulfide solvent (15 ml.) introduced six atoms of deuterium per molecule of decaborane. Each exchange was carried out for 90 hours at room temperature. The HCl–DCl gas mixtures were analyzed at equilibrium by an infrared method. After the third exchange reaction the hydrogen chloride content of the gas mixture was negligible and the recovered decaborane (9.5 mmole) analyzed[1] properly for $B_{10}H_8D_6$.

In the infrared this material exhibited B–H bridge, B–H terminal and B–D terminal stretching bands. No 7.30μ B–D bridge was observed.

(1) M. F. Hawthorne and J. J. Miller, THIS JOURNAL, **80**, 754 (1958).
(2) I. Shapiro, M. Lustig and R. E. Williams, *ibid.*, **81**, 838 (1959).