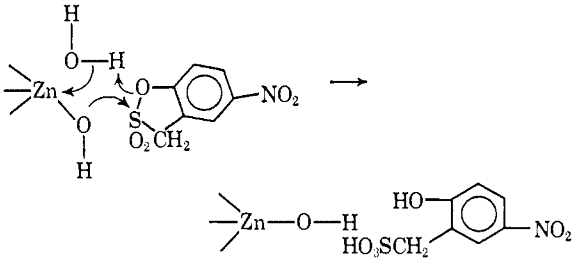
From the foregoing summary of our findings it seems clear that the mechanism of the carbonic anhydrase catalyzed hydrolysis of I must be very closely related to the mechanisms for the action of the enzyme on other types of substrates. A fundamental problem then in postulating a mechanism for the action of carbonic anhydrase on the sulfonate ester I is to decide what group on the enzyme with a p*K* of approximately 7 is responsible for the observed catalytic properties.

The suggestion has been made that a zinc-bound water molecule or hydroxide ion is directly transferred to carbon dioxide in the carbonic anhydrase catalyzed hydration of this substrate.[35] Two different types of mechanisms for the action of carbonic anhydrase on various substrates have evolved from this suggestion. On the one hand it has been postulated that the attack of the zinc-bound water on carbonic anhydrase substrates is assisted by a histidine residue with a p*K* of about 7 in the enzyme which acts in its basic form.[36] Alternatively, recent infrared studies on the action of carbonic anhydrase have been interpreted in terms of a mechanism which involves a zinc-bound hydroxide ion as the active species.[37,38] According to this hypothesis, then, the ionization of the zinc-bound water in carbonic anhydrase occurs with a p*K* of ~7.

On the basis of presently available evidence we cannot make a final judgment as to the relative merits of the alternative mechanisms. Certainly, at least in the case of HCAB[29] chemical modification of histidine residues can cause inhibition of enzymatic activity. However, we feel that there is no really compelling reason to invoke the direct participation of a histidine residue in the catalytic mechanism of carbonic anhydrase action. An aspect of the mechanisms postulating the involvement of a histidine residue as a general base which is particularly unattractive to us is that a protonated imidazolium species is formed in the step in which the zinc-bound water attacks the substrate. For the enzyme to regain its catalytic activity this imidazolium ion must lose its proton to the solvent. However, we know that proton transfer from the imidazolium ion to water occurs with a rate constant of approximately 10^3 sec^{-1},[39,40] a value which is below the maximum turnover number which has been found for carbonic anhydrase (turnover number $>10^4$ sec^{-1}).[41]

Indeed, it is because of the large magnitude of this maximum turnover number that we postulate the scheme shown below in eq 2 for the action of carbonic anhydrase in catalyzing the hydrolysis of I. In this scheme a zinc hydroxide complex is considered to be the catalytically active species. However, the cyclic reaction pathway of eq 2 avoids the difficulty of postulating a net proton transfer from the enzyme to the solvent, a reaction which may be too slow to be consistent with the high catalytic efficiency of carbonic anhydrase.[42]



(2)

Acknowledgment. The support of the National Institute of General Medical Sciences is gratefully acknowledged.

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(35) R. P. Davis, *Enzymes*, **5**, 545 (1961).

(36) Y. Pocker and D. R. Storm, *Biochemistry*, **7**, 1202 (1968).

(37) M. E. Riepe and J. H. Wang, *J. Amer. Chem. Soc.*, **89**, 4229 (1967).

(38) M. E. Riepe and J. H. Wang, *J. Biol. Chem.*, **243**, 2779 (1968).

(39) I. Amdur and G. G. Hammes, "Chemical Kinetics: Principles and Selected Topics," McGraw-Hill Book Co. Inc., New York, N. Y., 1966, p 185.

(40) M. Eigen, G. G. Hammes, and K. Kustin, *J. Amer. Chem. Soc.*, **82**, 3482 (1960).

(41) H. DeVoe and G. B. Kistiakowsky, *ibid.*, **83**, 274 (1961).

(42) The authors acknowledge the great value of their discussions with various participants at the First Conference on the Chemistry and Mechanism of Enzyme Action held in New Orleans in Dec 1968 in their formulation of the mechanism of eq 2. Obviously, mechanisms equivalent to that shown in eq 2 can be written for the carbonic anhydrase catalyzed hydration of carbon dioxide and the hydrolysis of nitrophenyl esters of carboxylic acids.

Communications to the Editor

Terpenoids. LXIV.[1] Chemical Studies of Marine Invertebrates. V.[2] The Isolation of Three New Holothurinogenins and Their Chemical Correlation with Lanosterol[3]

Sir:

Hydrolysis of the toxic glycosides[4] of various species of sea cucumbers (family *Holothurioideae*) has led to the isolation[5-8] of several sapogenins, which have been assumed to possess a lanostane skeleton (see, for instance, structure IV for griseogenin[6]) on the basis of extensive spectral and circumstantial chemical evidence.[5-8] We should now like to report[9] the isolation

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(1) For part LXIII see T. Nakano, M. Hasegawa, T. Fukumaru, L. J. Durham, H. Budzikiewicz, and C. Djerassi, *J. Org. Chem.*, in press.

(2) For part IV see W. Rudiger, W. Klose, B. Tursch, N. Houvenaghel-Crevecoeur, and H. Budzikiewicz, *Ann.*, **713**, 209 (1968).

(3) The work at Stanford University was supported by Grant GM 06840 from the National Institutes of Health, while the investigations in Brussels were aided by the Centre Belge d'Océanographie and the I.R.S.I.A.

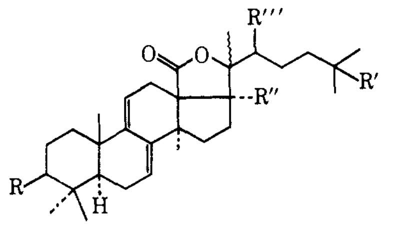
(4) For leading references see J. D. Chanley, R. Ledeen, J. Wax, R. F. Nigrelli, and H. Sobotka, *J. Am. Chem. Soc.*, **81**, 5180 (1959); S. Shimada, *Science*, **163**, 1462 (1969).

(5) J. D. Chanley, T. Mezzetti, and H. Sobotka, *Tetrahedron*, **22**, 1857 (1966).

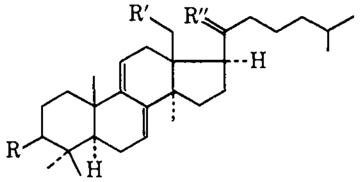
(6) B. Tursch, I. S. de Souza Guimarães, B. Gilbert, R. T. Aplin, A. M. Duffield, and C. Djerassi, *ibid.*, **23**, 761 (1967).

(7) J. D. Chanley and C. Rossi, *ibid.*, **25**, 1897 (1969).

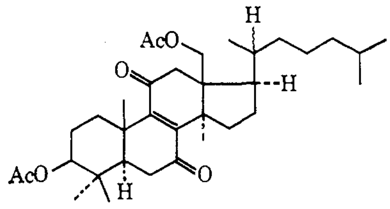
(8) J. D. Chanley and C. Rossi, *ibid.*, **25**, 1911 (1969).



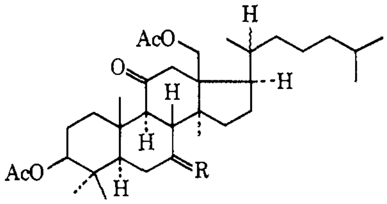
I, R = OH, R' = OCH_3; R'', R''' = H
II, R, R' = OH; R'', R''' = H
III, R = OH; R', R'', R''' = H
IV, R, R'', R''' = OH; R' = H
V, R = OAc; R' = OH; R'', R''' = H
VI, R = OAc; R', R'', R''' = H



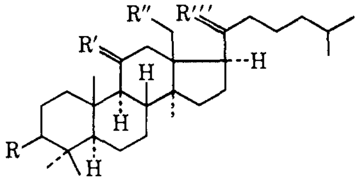
VII, R = R' = OH; R'' = $\left\langle\begin{smallmatrix}CH_3\\ OH\end{smallmatrix}\right.$
VIII, R = R' = OAc; R'' = $\left\langle\begin{smallmatrix}CH_3\\ OH\end{smallmatrix}\right.$
IX, R = R' = OAc; R'' = CH_2
X, R = R' = OAc; R'' = $\left\langle\begin{smallmatrix}CH_3\\ OH\end{smallmatrix}\right.$



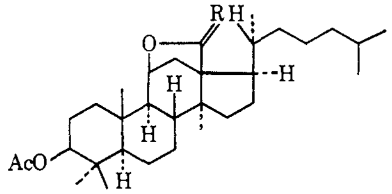
XI



XII, R = O
XIII, R = $\begin{smallmatrix}S\\ S\end{smallmatrix}\Big]$



XIV, R, R'' = OAc; R' = O; R''' = $\left\langle\begin{smallmatrix}CH_3\\ H\end{smallmatrix}\right.$
XV, R, R'' = OAc; R' = O; R''' = $\left\langle\begin{smallmatrix}H\\ CH_3\end{smallmatrix}\right.$
XVI, R, R'' = OH; R' = $\left\langle\begin{smallmatrix}OH\\ H\end{smallmatrix}\right.$; R''' = $\left\langle\begin{smallmatrix}CH_3\\ H\end{smallmatrix}\right.$
XVII, R = OAc; R' = $\left\langle\begin{smallmatrix}OH\\ H\end{smallmatrix}\right.$; R'' = H; R''' = $\left\langle\begin{smallmatrix}CH_3\\ H\end{smallmatrix}\right.$



XVIII, R = H_2
XIX, R = O

of three new holothurinogenins, ternaygenin (I, $C_{31}H_{48}O_4$, mp 239–242°, $[\alpha]_D$ +2°), koellikerigenin (II, $C_{30}H_{46}O_4$, mp 213–214°, $[\alpha]_D$ −8°), and seychellogenin (III, $C_{30}H_{46}O_3$, mp 234–238°, $[\alpha]_D$ −7°), from the sea cucumber *Bohadschia koellikeri*,[10] which could be related chemically to lanosterol. This constitutes the first unambiguous demonstration for the structure of the holothurinogenins and thus supports indirectly also the structural assignments of those members[5–8] possessing a C-17 hydroxyl function.

All three compounds exhibited infrared ($\gamma_{max}^{CHCl_3}$ 1755–1765 cm^{-1}) and ultraviolet (λ_{max}^{EtOH} 237, 244, 252 nm (ϵ 11,000, 12,000, 8000) absorptions typical for γ-lactones and heteroannular dienes. Their nmr spectra exhibited seven methyl signals, of which two were shifted to lower field in I and II as compared to III, indicating the proximity of an oxygen function (at C-25). Interconversion among the three sapogenins was effected as follows. Acetylation of koellikerigenin (II) produced the 3-monoacetate V (mp 213–216°), which was dehydrated with phosphorus oxychloride in pyridine and hydrogenated (platinum on $BaSO_4$ in ethyl acetate) to yield seychellogenin acetate (VI) (mp 211–213°). Direct treatment of koellikerigenin (II) with methanol in the presence of hydrochloric acid—under conditions employed in the hydrolysis of the sea cucumber glycosides—afforded ternaygenin (I), thus suggesting that the latter might be an artefact produced during hydrolysis of the saponin.

Seychellogenin (III) is the simplest known holothurinogenin and hence was selected for chemical correlation with lanosterol. Reduction with lithium aluminum hydride provided the triol VII ($C_{30}H_{50}O_3$, mp 172–173°), whose 3,18-diacetate VIII ($C_{34}H_{54}O_5$, mp 139–144°, $[\alpha]_D$ +67°) was dehydrated with phosphorus oxychloride. All three double bond isomers ($\Delta^{17(20)}$, $\Delta^{20(21)}$, and $\Delta^{20(22)}$) were separated by preparative tlc and the major isomer IX ($C_{34}H_{52}O_4$, mp 130–131°, nmr; two-hydrogen broad doublet at δ 4.80) was hydrogenated (PtO_2 in ethyl acetate). The two C-20 epimers of X ($C_{34}H_{54}O_4$, mp 97–105°) were not separated, but were carried through the traditional sequence[11] of chromium trioxide oxidation to the Δ^8-7,11-enedione XI (λ_{max}^{EtOH} 271.5 nm (ϵ 7100)), zinc reduction to XII, 7-monothioethylene ketal (XIII) formation, and desulfurization with Raney nickel. At this stage, the two C-20 epimers XIV ($C_{34}H_{56}O_5$, mp 140.5–143°, $[\alpha]_D$ +38°) and XV (mp 130–131°, $[\alpha]_D$ +53°) were separated by preparative tlc and the latter

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(9) The empirical formulas of all compounds were established by high-resolution mass spectrometry and all rotations were measured in chloroform solution.

(10) Collected by B. T. in Aug 1966 in the Seychelles Archipelago in the Indian Ocean.

(11) W. Voser, M. Montavon, H. H. Günthard, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta*, 33, 1893 (1950).

reduced with lithium aluminum hydride to yield the hitherto undescribed lanostane-3β,11β,18-triol (XVI, $C_{30}H_{54}O_3$, mp 228–229°, $[\alpha]_D$ +43°). An authentic specimen of XVI was prepared as follows.

Oxidation of 3β-acetoxylanostan-11β-ol (XVII)[11] with lead tetraacetate and iodine in cyclohexane solution under illumination[12] yielded a complex mixure, which was directly reduced with lithium aluminum hydride and then subjected to successive column and thin layer chromatography to yield, after acetylation, a mixture of 11,18 (XVIII) and 11,19[12] ethers. Chromium trioxide oxidation of this mixture followed by chromatography led in 21% yield to the 11,18-lactone XIX ($C_{32}H_{52}O_4$, mp 222–222.5°, $\nu_{\max}^{\mathrm{CHCl_3}}$ 1760 and 1735 cm^{-1}), which upon reduction with lithium aluminum hydride afforded the triol XVI (mp 227–229°, $[\alpha]_D$ +43°). Identity with the sample derived from seychellogenin (III) was established by mixture melting point determination, identity of the nmr, mass, and infrared spectra, as well as identical tlc mobility. The present correlation of seychellogenin (III) with lanosterol establishes rigorously all structural and stereochemical features of the three holothurinogenins I II, and III with the exception of the stereochemistry at C-20.

[12] We employed a modification of the procedure of D. H. R. Barton, R. P. Budhiraja, and J. F. McGhie, *J. Chem. Soc., C*, 336 (1969), who recorded the isolation of one ether to which the structure of 11β,19-oxylanostan-3β-ol was assigned.

Peter Roller, Carl Djerassi
Department of Chemistry, Stanford University
Stanford, California 94305

Rudolphe Cloetens, B. Tursch
Services de Chimie Alicyclique et d'Anatomie Comparée
Université Libre de Bruxelles, Brussels, Belgium

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Five-Coordinate Nickel(III). The Structure of $NiBr_3(P(CH_3)_2(C_6H_5))_2 \cdot 0.5NiBr_2(P(CH_3)_2(C_6H_5))_2 \cdot C_6H_6$

Sir:

Examples are known where a nickel complex contains either two different stereochemical arrangements for a fixed coordination number[1] *or* two different coordination numbers.[2] This communication adds a third possibility and reports the X-ray structural determination of a compound that contains simultaneously two different *oxidation states* of nickel, two coordination numbers, and two unrelated stereochemistries. In so doing it provides the first details of the stereochemistry of a Ni(III) complex.

Although trivalent nickel has been proposed in complexes with a variety of donor atoms,[3,4] evidence for the d^7 configuration is largely limited to the observation of magnetic moments in the range 1.7–2.1 BM, consistent with low-spin Ni(III).[5] On the basis of a zero dipole moment and μ_{eff} = 1.91 BM, Jensen, *et al.*,[6] reformulated $NiBr_3(P(C_2H_5)_3)_2$ as trigonal-bipyramidal rather than square-pyramidal Ni(III), as they had suggested earlier.[7] Van Hecke and Horrocks[8] observed esr signals on powdered samples of $NiBr_3((C_6H_5)_2P(CH_2)_nP(C_6H_5)_2)$ (n = 2, 3), but they were unable to interpret the electronic spectra in terms of a five-coordinate complex. Gray and coworkers[9,10] have recently questioned the representation of $[Ni(mnt)_2]^-$ and $[Ni(diars)_2Cl_2]^+$ (mnt^{2-} and diars are $(CN)_2C_2S_2{}^{2-}$ and o-$C_6H_4(As(CH_3)_2)_2$, respectively) as Ni(III) complexes, so obviously additional data are needed to substantiate the existence of trivalent nickel in molecular complexes.

Measurements on the position of equilibrium between four- and five-coordinate nickel(II) complexes led us to anticipate that pentacoordination might also be achieved by oxidation of the square-planar $NiBr_2(P(CH_3)_2(C_6H_5))_2$.[11] In fact, adding a benzene solution of bromine to a dark red solution of $NiBr_2(P(CH_3)_2(C_6H_5))_2$ gives an intensely blue solution from which we isolated a bluish black crystalline solid whose elemental analysis corresponds to the formulation $NiBr_3(P(CH_3)_2(C_6H_5))_2$ (I). *Anal.* Calcd for $C_{16}H_{22}Br_3NiP_2$: C, 33.40; H, 3.86; Br, 41.73; P, 10.80; Ni, 10.22. Found: C, 33.58; H, 3.94; Br, 41.96, 41.19; P, 10.78; Ni, 10.55. Although the solid is stable in air, solutions of I decompose slowly, thereby hindering the physical measurements, but the qualitative values indicate that I is a nonelectrolyte in nitromethane and a monomer in chloroform. The magnetic moment (μ_{eff} = 2.17 BM at 296°K) falls within the range expected for low-spin, pentacoordinate d^7 complexes.[5] In dichloromethane solutions, electronic absorption maxima have been recorded at 17,000, 20,800, 25,600, 30,300, and 37,000 cm^{-1} with ϵ values in the 3800–13,000 range. The spectrum of I in the solid state is very similar to the solution spectrum. An assignment of the spectra appears to be consistent with that expected for a Ni(III) complex and will be reported with those of other $NiBr_3L_2$ complexes.

Attempts to obtain crystals of I for a structure determination led to isolation of suitable crystals from a benzene–hexane solution. The material was found to crystallize in space group $C_i{}^1$-$P\bar{1}$ of the triclinic system, in a unit cell of dimensions a = 9.021 (5), b = 17.951 (10), c = 11.181 (6) Å; α = 98° 52 (1)′, β = 94° 29 (1)′, and γ = 90° 44 (1)′. The observed density of 1.67 ± 0.02 g/cm^3 corresponds to 3.1 molecules of I in the cell. Since this would require one molecule of I to have a center of symmetry, further measurements were made on the recrystallized sample. The magnetic susceptibility and electronic spectra of this recrystallized sample, II, suggested a stoichiometric ratio of one diamagnetic $NiBr_2(P(CH_3)_2(C_6H_5))_2$ molecule for every two paramagnetic molecules of I. The X-ray structure determined using diffractometer data subsequently confirmed the existence of the 1:2 stoichiometry, in addition to the presence of two molecules of solvent benzene, and resulted in the first structural determination of an authentic Ni(III) complex.

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[1] B. T. Kilbourn, H. M. Powell, and J. A. C. Darbyshire, *Proc. Chem. Soc.*, 207 (1963).

[2] A. Kircheiss and R. Karutz, *Z. Chem.*, **8**, 471 (1968).

[3] N. F. Curtis and D. F. Cook, *Chem. Commun.*, 962 (1967), and references contained therein.

[4] A. H. Maki, N. Edelstein, A. Davison, and R. H. Holm, *J. Am. Chem. Soc.*, **86**, 4580 (1964).

[5] G. Dyer and D. W. Meek, *ibid.*, **89**, 3983 (1967); B. N. Figgis and J. Lewis, *Progr. Inorg. Chem.*, **6**, 196 (1964).

[6] K. A. Jensen, B. Nygaard, and C. T. Pedersen, *Acta Chem. Scand.*, **17**, 1126 (1963).

[7] K. A. Jensen and B. Nygaard, *ibid.*, **3**, 474 (1949).

[8] G. R. Van Hecke and W. D. Horrocks, Jr., *Inorg. Chem.*, **5**, 1968 (1966).

[9] E. I. Stiefel, J. H. Waters, E. Billig, and H. B. Gray, *J. Am. Chem. Soc.*, **87**, 3016 (1965).

[10] P. Kreisman, R. Marsh, J. R. Preer, and H. B. Gray, *ibid.*, **90**, 1067 (1968).

[11] E. C. Alyea and D. W. Meek, *ibid.*, in press.