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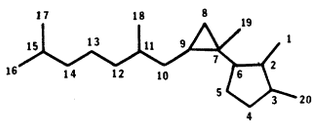
23. These research activities were performed from the University of Miami's R.V. *Calanus* in numerous habitats of the Bahama Islands. *Calanus* operations were supported by the National Science Foundation.
24. Extracts of two *Halimeda* species, less common in our study sites in the Bahamas, *H. goreauii* Taylor and *H. lacrimosa* var. *globosa* Dawes, contained little if any halimedatrial but did contain new, as yet unknown, terpenoids.
25. Halimedatrial showed infrared absorptions at 1720, 1690, and 1657 cm^{-1} for the saturated aldehyde, cyclopropyl aldehyde, and unsaturated aldehyde carbonyl groups. Ultraviolet absorbance at 244 nm (ϵ = 11,400) confirmed the latter infrared band to correspond to an α,β-unsaturated aldehyde. We suggest the name "halimedane" to describe this new diterpene ring system, and the numbering sequence provided in **3**.



26. The spectral features of triacetate **2** were recorded as follows: $[\alpha]_D^{27} = +13.3°$ (c = 0.9, $CHCl_3$); mass spectrum: M^+ −HOAc m/z 386.2447, M^+ −2 HOAc m/z 326.2235, M^+ −3 HOAc. m/z 266.2028, IR: 2990, 1720, 1450, 1390, 1240 cm^{-1}, ^1H NMR (360 MHz, benzene-d_6): δ 5.55 (1 H, bs), 5.18 (1 H, t, J = 7), 4.99 (1 H, d, J = 8), 4.64 (2 H, s), 4.10 (1 H, dd, J = 12, 6), 4.0 (1 H, dd, J = 12, 6), 4.0 (1 H, d, J = 15), 3.85 (1 H, d, J = 15), 3.08 (1 H, m), 2.42 (1 H, dd, J = 15, 9), 2.23 (1 H, dd, J = 15, 2), 2.13 (1 H, t, J = 7), 2.07 (1 H, m), 1.78 (1 H, dd, J = 9, 4), 1.76 (3 H, s), 1.70 (3 H, s), 1.67 (3 H, s), 1.67 (3 H, s), 1.61 (3 H, s), 1.55 (3 H, s), 1.50 (1 H, m), 0.83 (1 H, dd, J = 9), 0.44 (1 H, dd, J = 5), ^{13}C-NMR (50 MHz, $CDCl_3$): δ 171.0 (s), 170.9 (s), 170.7 (s), 138.7 (s), 138.0 (s), 131.4 (s), 131.0 (d), 124.0 (d), 122.4 (d), 69.0 (t), 66.0 (t), 62.0 (t), 48.9 (d), 45.0 (d), 39.5 (t), 34.9 (t), 28.7 (s), 26.5 (t), 25.9 (q), 21.8 (d), 3x 21.0 (q), 19.7 (t), 17.7 (q), 16.6 (q).
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28. The concentration used, 2000 ppm, represents the average concentration of halimedatrial on a dry weight basis. Details of similar toxicity and feeding avoidance bioassays have been described [V. J. Paul, O. J. McConnell, W. Fenical, *J. Org. Chem.* **45**, 3401 (1980)].
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31. We thank Dr. J. N. Norris, Smithsonian Institution, for taxonomic studies and identification of various *Halimeda* collections. (All voucher specimens are deposited in the Algal Collection, U.S. National Herbarium.) We thank Drs. J. N. Norris, M. M. Littler, J. C. Ogden, M. E. Hay, L. Hillis-Colinuaux, and E. A. Shinn for critical reading of this manuscript. Supported by NSF grant CHE81-11907.

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Free-Metal Ion Depletion by "Good's" Buffers

Abstract. *Metal-ion affinity (formation) constants were determined for two "Good's" buffers,* N-*tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) and* N,N-*bis(2-hydroxyethyl)glycine (bicine). The metal chelates formed undergo loss of an internal ligand (alcohol) proton (bicine) and undergo hydrolysis (bicine and TES) and dimerization reactions (TES). Bicine and TES buffer not only hydrogen ions but also metal ions. The metal complexes of "Good's" buffers also buffer hydrogen ions by secondary reactions. The consequences of these reactions are considered in relation to biomedical research.*

In 1966, Good *et al.* reported (*1*) a new series of buffers for use in biological research. The buffers had several important properties including a pK_a (the negative logarithm of the acid dissociation constant) between 6 and 8, maximum water solubility with minimum solubility in other solvents, minimum salt effects, low ability to cross biological membranes, and supposedly low affinities for biologically important metal ions. It is the last of these properties that is of concern here.

The widespread use of "Good's" buffers in biological studies involving metal ions, for example, the calculation of protein-metal affinity constants, probably is in part responsible for the many conflicting data and conclusions derived by investigators studying identical metal cation–protein systems at the same pH in carefully executed experiments. For example, if one is studying the binding of Co^{2+} to an apoenzyme at pH 7.3 in an ADA {[(carbamoylmethyl)imino]diacetic acid} buffer, the actual reaction under consideration is

$$Co(ADA)_2^{2-} + \text{apoenzyme} \rightleftharpoons 2ADA^{2-} + \text{M-enzyme complex} \quad (1)$$

The same reaction studied in a BES {2-[bis(2-hydroxyethyl)amino]ethanesulfonic acid} buffer is

$$Co(BES)^{+} + \text{apoenzyme} \rightleftharpoons BES^{-} + \text{M-enzyme complex} \quad (2)$$

Equations 1 and 2 will yield very different metal cation–protein affinity constants. This conclusion is based on metal-buffer stability constants and ligand–metal ion stoichiometries that have been determined in this laboratory. The metal cation–protein binding constants will be dependent on the buffer used. Correct affinity constant values for Eq. 3

$$M^{x+} + \text{apoenzyme} \overset{K_f}{\rightleftharpoons} \text{M-enzyme complex} \quad (3)$$

could be obtained in the presence of various buffers if the appropriate M-buffer formation constants (K_B) were known. From Eqs. 4 and 5,

$$M^{x+} + \text{buffer}^{y-} \overset{K_B}{\rightleftharpoons} M(\text{buffer})^{(x-y)+} \quad (4)$$

$$M(\text{buffer})^{(x-y)+} + \text{apoenzyme} \overset{K'_f}{\rightleftharpoons} \text{M-enzyme complex} + \text{buffer}^{y-} \quad (5)$$

(where K_B is the equilibrium constant of the buffer), it is obvious that

$$K_f = K'_f K_B \quad (6)$$

Furthermore, the metal ion interaction of various "Good's" buffers can be quite complex, and Eq. 6 represents only the most simple case, that is, no hydroxo complex formation (Eq. 7)

$$\text{M-buffer}^{x+} + OH^{-} \rightleftharpoons \text{M-buffer}\,(OH)^{(x-1)+} \quad (7)$$

no 2:1 or higher complex formation of buffer to metal ion (Eq. 8),

$$M(\text{buffer})^{x+} + \text{buffer}^{y-} \rightleftharpoons M(\text{buffer})_2^{(x-y)+} \quad (8)$$

and no ionization of "internal" buffer protons (Eq. 9)

$$M(\text{buffer})^{x+} \rightleftharpoons M(H_{-1}\ \text{buffer})^{(x-1)+} + H^{+} \quad (9)$$

We have obtained data (*2*) that reactions such as those in Eqs. 7, 8, and 9 do occur with "Good's" buffer-metal complexes in the pH range 6 to 9 commonly used in biological and medical research.

Table 1 presents metal ion affinity (formation) constants (*3*) for bicine [*N,N*-bis(2-hydroxyethyl)glycine] and TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid], two of the "Good's" buffers. These buffers were chosen to emphasize that "Good's" compounds buffer H^+ concentration as well as metal ion concentrations and also that the resultant metal complexes buffer H^+ (Eqs. 7 and 9). Bicine forms 1:1 and 2:1 buffer–metal ion complexes with Cu(II), Co(II), and Zn(II), and only 1:1 complexes with Mn(II) and Ca(II). The bis(bicinato)copper(II) chelate undergoes a most remarkable reaction, dechelation of an aminoacidate group upon alcohol proton ionization (Eq. 10) (*4*)

$$Cu(\text{bicine})_2 \overset{K_{1a}}{\rightleftharpoons} Cu(H_{-1}\ \text{bicine})H_2O + \text{bicine}^{-1} + H^{+} \quad (10)$$

This reaction is also followed by deprotonation of the coordinated H_2O to produce the corresponding hydroxo complex (K_{OH}) (Eq. 11)

$$Cu(H_{-1}\ \text{bicine})H_2O \overset{K_{OH}}{\rightleftharpoons} Cu(H_{-1}\ \text{bicine})OH^{-} + H^{+} \quad (11)$$

Table 1. Formation (affinity) constants.

Ion	Bicine		TES, log K_{1B}
	log K_{1B}	log K_{2B}	
H^+	8.39 ± 0.01		7.60 ± 0.01
Cu(II)	8.07 ± 0.01	5.40 ± 0.02*	3.90 ± 0.02†
Co(II)	5.30 ± 0.01	3.38 ± 0.02	2.07 ± 0.02
Zn(II)	5.37 ± 0.01	2.67 ± 0.03	2.08 ± 0.02
Mn(II)	3.02 ± 0.01		‡
Ca(II)	2.66 ± 0.01		~ 0

*See Eqs. 10 and 11. †See Eqs. 12 and 13. ‡Hydrolysis and precipitation are concomitant with any complex formation.

The values for K_{1a} and K_{OH} are $10^{-12.36 \pm 0.02}$ and $10^{-10.42 \pm 0.02}$, respectively. On the other hand, TES forms much weaker metal complexes with metal ions (no complex formation with Ca^{2+}) than bicine. However, the 1:1 Cu(II)–TES chelate undergoes hydrolysis (Eq. 12) and subsequent dimerization (K_D) (Eq. 13) in the neutral *p*H range (*5*)

$$Cu(TES)^+ \overset{K_{OH}}{\rightleftharpoons} Cu(TES)OH + H^+ \quad (12)$$

$$2Cu(TES)OH \overset{K_D}{\rightleftharpoons} Cu_2(TES)_2(OH)_2 \quad (13)$$

The values for K_{OH} and K_D are $10^{-6.62 \pm 0.02}$ and $10^{+2.30 \pm 0.04}$, respectively. It is obvious from the above reactions that not only do "Good's" buffers complex metal ions but also that the metal complexes formed have a rich solution chemistry. These latter reactions further buffer H^+ concentration (Eqs. 10 through 12) and strengthen the metal complexes formed (Eq. 13).

Although the constants listed in Table 1 are on the whole small compared to those of metal ions for active sites of apoenzymes (10^{10} to 10^{20}), they are of sufficient strength to lead workers to erroneous conclusions in systems of low metal-binding capabilities, for example, fluorescence quenchers, spin labels, and paramagnetic shift reagents. Furthermore, the "Good's" buffer ADA forms a very stable chelate with Cu(II) ($K_1 = 10^{10.57}$)[2], rivaling that of apoenzymes, and Ca(II) ($K_1 = 10^{4.01}$)[2]. The latter constant is high enough to cause the extraction of labile Ca(II) from enzymes or other biochemicals, thereby altering the structure of the protein with concomitant loss of activity.

The users of "Good's" buffers should no longer assume, as is generally believed, that they do not complex metal ions below or at physiological *p*H values. Studies on the following "Good's" buffers, ACES {2-[(2-amino-2-oxoethyl)amino]ethanesulfonic acid}, ADA, BES, bicine, and TES indicate that all complex metal ions. The general tendency to bind M(II) is in the order ADA > bicine > ACES > BES ~ TES. The last three of these buffers, however, do not bind Ca(II). Those doing studies in the presence of these buffers and metal ions should take into account the metal complexes formed and their possible deleterious effects on the interpretation of data collected in their presence. Routinely used buffers other than those developed by Good *et al.* (*1*) have also been shown to complex metal ions, most notable tris, [tris(hydroxymethyl)methylamine] (*6*).

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3. Potentiometric formation curves were obtained at 25.00° ± 0.05°C and 0.1*M* (KNO_3) ionic strength on a Corning Digital 130 research model *p*H meter. Metal ion concentrations were ~ 0.025*M*.
4. Visible spectra (not shown) of 2:1 bicine–Cu(II) solutions from **a** = 0 to **a** = 2.0 (**a** is the number of moles of base per mole of complexing agent) evince a monotonic shift in maximum wavelength (λ_{max}) and extinction coefficient (ϵ_{max}) from **a** = 0.0 to 1.0, indicating the formation of [Cu(bicine)$_2$]. The λ_{max} value at **a** = 1.0 is 610 nm, in good accord with the value for a variety of other bis(aminoacidate)copper(II) chelates ($\lambda_{max} \simeq 605$ nm [R. Nakon, E. Beadle, Jr., R. J. Angelici, *J. Am. Chem. Soc.* **96**, 719 (1974)]. From **a** = 1.0 to 2.0, λ_{max} shifts monotonically to lower energy (λ_{max} = 724 nm at **a** = 2.0); this behavior indicates the loss of an aminoacidate ligand (Eq. 10). The λ_{max} and ϵ_{max} values of 1:1 and 2:1 bicine–Cu(II) solutions at **a** = 3.0 and 2.0, respectively, are 724 nm and 64 M^{-1} cm^{-1}, an indication that both solutions contain the same species, [Cu(H_{-1} bicine)OH^-]. The electron spin resonance spectra of 1:1 and 2:1 bicine—Cu(II) solutions at **a** = 3.0 and 2.0, respectively, are identical; similarly, at **a** = 1.0 and 0.5, respectively, identical spectra were observed. However, the 2:1 spectrum at **a** = 1.0 was never seen in any 1:1 spectrum from **a** = 0.0 to 3.0. The species at **a** = 3.0 (2.0) is [Cu(H_{-1} bicine)OH^-], at **a** = 1.0 (0.5) is [Cu(bicine)$^+$], but at **a** = 1.0 (2:1 system) is [Cu(bicine)$_2$].
5. Visible spectral data of 1:1 TES–Cu(II) solutions show no large blue shift until *p*H > 11, an indication that there are no alcohol deprotonation reactions in the acidic or neutral *p*H regions.
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Nucleotide Sequence of a Light Chain Gene of the Mouse I-A Subregion: $A\beta^d$

Abstract. *Ia (I region–associated) antigens are cell-surface glycoproteins involved in the regulation of immune responsiveness. They are composed of one heavy (α) and one light (β) polypeptide chain. We have sequenced the gene encoding the $A\beta^d$ chain of the BALB/c mouse. The presence of six exons is predicted by comparison with the complementary DNA sequences of human β chains and with partial protein sequence data for the $A\beta^d$ polypeptide. Sequence comparisons have been made to other proteins involved in immune responses and the consequent implications for the evolutionary relationships of these genes are discussed.*

The major histocompatibility complex (MHC) of the mouse is a cluster of genes encoding at least three different classes of proteins involved in immune responses (*1*). Class I molecules, the classic transplantation antigens, and class II molecules are cell-surface, membrane-bound glycoproteins. Class III molecules are serum protein components of the complement pathway. While class I proteins are found on essentially all cells, class II representatives appear limited mainly to the surface of B cells and to antigen-presenting cells such as macrophages. Class II proteins are required for antigen presentation and lymphocyte interactions involved in the activation and differentiation of antibody-producing cells or B cells (*2*). Murine class II genes are located in the I (immune response) region of the MHC and are consequently referred to as I region–associated or Ia antigens (*3*).

Two types of Ia antigen have been defined in mice, I-A and I-E. Both are composed of two noncovalently linked polypeptides, a heavy chain (α) of about 34,000 daltons and a light chain (β) of about 28,000 daltons (molecular size). In the past 2 years, advances in protein microsequencing and recombinant DNA technologies have led to a definition of the primary structure of various human and murine class II molecules (*4–14*). Both α and β polypeptides may be divided into two external domains of approxi-