References and Notes

1. R. H. Johnson, J. Northrop, R. A. Eppley, *J. Geophys. Res.* **68**, 4251 (1963).
2. R. H. Johnson, *Bull. Seismol. Soc. Amer.* **56**, 109 (1966).
3. Located by the U.S. Coast and Geodetic Survey.
4. H. W. Menard, *Science* **155**, 72 (1966).
5. D. A. McManus, *Bull. Geol. Soc. Amer.* **78**, 527 (1967).
6. L. R. Sykes, *J. Geophys. Res.* **72**, 1231 (1967).
7. P. Dehlinger, R. W. Couch, M. Gemperle, *ibid.*, p. 1233.
8. H. Benioff, *Bull. Seismol. Soc. Amer.* **41**, 31 (1955).
9. J. N. Brune, *J. Geophys. Res.* **73**, 777 (1968).
10. T. M. Atwater and J. D. Mudie, *Science* **159**, 729 (1968).

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Magnesium Sulfate Interactions in Seawater from Solubility Measurements

Abstract. *The extent of association between magnesium and sulfate ions was determined in artificial seawater by a solubility technique. About 10 percent of the magnesium ions were found to be associated. This result supersedes the earlier value found in this laboratory and agrees with the results of Garrels and Thompson, Thompson, and Fisher.*

Several researchers determined the extent of association between magnesium and sulfate ions in seawater. Garrels and Thompson, Thompson, and Fisher (*1*) found that between 9 and 11 percent of the magnesium ions are associated to sulfate ions. The results of Pytkowicz *et al.* (*1*) indicated that the extent of association was greater. In the present work we redetermined the extent of association, again by a method based on the solubility of brucite, and obtained results that agree with those of the other workers. The present procedure, in contrast to our previous one, avoided both the use of a value for the thermodynamic solubility product of brucite and the possibility of carbonate coprecipitation. Particle size effects were minimized because a comparative technique was used.

The procedure was as follows. Two solutions, with compositions shown in Table 1, were equilibrated with 40- to 60-mesh white platy Quebec brucite and the steady-state *p*H was measured at 25° ± 0.1°C. The *p*H measurements were made with an Orion model 801 digital *p*H meter, with a precision of ± 0.002 *p*H units. Solution B, with the exception of carbonate and borate which were not added in its preparation, corresponds to seawater of 34.8 parts per mille salinity. The absence of carbonate in the two solutions was necessary to avoid either the precipitation of calcium carbonate which could affect the final *p*H or the possible alteration of the brucite surface by carbonate inclusion. Fresh samples of brucite were prepared and washed with distilled water before each pair of runs in solutions A and B. Preliminary measurements had shown that the same final *p*H was obtained either with immersion of the glass electrode into the brucite or with stirring of the solution and of the solid. The immersion technique was used in subsequent experiments because it permitted faster equilibration.

The calculations were based on the following equations. The thermodynamic solubility product of brucite is the same in solutions A and B. Therefore:

$$(a_{Mg})_A\,(a_{OH})^2_A = (a_{Mg})_B\,(a_{OH})^2_B \qquad (1)$$

For a given solution, the following equation, discussed by Pytkowicz *et al.* (*1*), is valid:

$$a = f_F\,(F) = f_T\,(T) \qquad (2)$$

where a is the activity, f is the activity coefficient, the parentheses represent molalities, F indicates free quantities, and T indicates total (free plus associated) quantities. From Eqs. 1 and 2 it follows that

$$\frac{(a_{OH})^2_A}{(a_{OH})^2_B} = \frac{(f_{Mg})_{BF}}{(f_{Mg})_{AF}}\,\frac{(Mg^{2+})_{BF}}{(Mg^{2+})_{AF}} \qquad (3)$$

The subscripts AF and BF represent the free ions in solutions A and B respectively.

Solutions A and B had the same stoichiometric ionic strength, that is, the stoichiometric ionic strength corrected for ion association. For solution A, which consists only of chlorides, there should be no ion association (*2*). For solution B the extent of association was estimated from the model of Garrels and Thompson (*1*). An assumption in the ion association model is that free activity coefficients depend only on the effective ionic strength and not on the composition of the medium. The adequacy of this assumption was verified by Pytkowicz and Kester (*3*) for sodium ions upon replacement of chloride by sulfate ions at constant effective ionic strength. It is likely, therefore, that $(f_{Mg})_{BF} = (f_{Mg})_{AF}$.

The absence of chloride ion association means that $(Mg^{2+})_{AF} = (Mg^{2+})_{AT}$, where the subscript AT refers to the total concentration in solution A. Also, the thermodynamic dissociation constant of water is the same in the two solutions. Therefore, Eq. 3 can be reduced to

$$(Mg^{2+})_{BF} = (Mg^{2+})_{AT}\,\frac{(a_H)^2_B}{(a_H)^2_A} \qquad (4)$$

Thus, $(Mg^{2+})_{BF}$, the concentration of free magnesium ions in solution B, may be obtained from the stoichiometric concentration of magnesium and from the values of the steady-state *p*H at equilibrium with brucite in solutions A and B. The concentration of $MgSO^0_4$ ion pairs is obtainable from

$$(MgSO^0_4)_B = (Mg^{2+})_{BT} - (Mg^{2+})_{BF} \qquad (5)$$

BT indicates the total concentration in solution B.

Table 1. Ionic concentrations of the solutions.

Ion	Concentration (molal units)	
	Solution A	Solution B
Na	0.473	0.482
Mg	.0548	.0548
Ca	.0106	.0106
K	.0102	.0102
Sr		.00009
Cl	.614	.564
SO_4		.0291
Br		.00086
F		.00005

Table 2. Steady-state *p*H, free magnesium ion concentration, concentration of $MgSO_4^0$ ion pairs, and percent of magnesium ions associated to sulfate ions at 25°C and 34.8 per mille salinity.

Run	Steady-state *p*H		$(Mg^{2+})_{BF}$	$(MgSO_4^0)$	Associated magnesium (percent)
	Solution A	Solution B			
1	9.314	9.335	0.0493	0.0055	10
2	9.287	9.314	.0482	.0066	12
3	9.308	9.328	.0499	.0049	9
Average					10

The results are presented in Table 2. We found that about 10 percent of the magnesium ions are associated with sulfate ions. This result is in good agreement with those of Garrels and Thompson, Thompson, and Fisher (*1*) which were obtained by a variety of methods. The values of the steady-state *p*H obtained in the present work are lower than those obtained earlier by Pytkowicz *et al.* (*1*).

The apparent (quasi-stoichiometric) solubility product of brucite in solution B, $K'_{SP} = (Mg^{2+})_T(a_{OH})^2$, was found to be $(2.4 \pm 0.2) \times 10^{-11}$. The thermodynamic solubility product may be expressed as $K_{SP} = (f_{Mg})_F(Mg^{2+})_F(a_{OH})^2$. According to Garrels and Thompson (*1*), $(f_{Mg})_F = 0.36$. Therefore, from our values of $(Mg^{2+})_F$ and a_{OH}, $K_{SP} = (7.8 \pm 0.5) \times 10^{-12}$. This result is only approximate because a_{OH} obtained from *p*H measurements is not exactly the thermodynamic activity of hydroxyl ions. However, the arbitrariness in the operational definition of *p*H does not detract from its practical value in self-consistent potentiometric studies of seawater (*4*). Our result is in good agreement with that of Hostetler (*5*), who found that $K_{SP} = (7.1 \pm 0.5) \times 10^{-12}$.

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References and Notes

1. R. M. Garrels and M. E. Thompson, *Amer. J. Sci.* **260**, 57 (1962); R. M. Pytkowicz, I. W. Duedall, D. N. Connors, *Science* **152**, 640 (1966); M. E. Thompson, *ibid.* **153**, 866 (1966); F. Fisher, *ibid.* **157**, 823 (1967).
2. C. W. Davies, *Ion Association* (Butterworths, London, 1962), p. 77.
3. R. M. Pytkowicz and D. R. Kester, *Amer. J. Sci.*, in press.
4. R. M. Pytkowicz, D. R. Kester, B. C. Burgener, *Limnol. Oceanogr.* **11**, 417 (1966).
5. B. B. Hostetler, *Amer. J. Sci.* **261**, 238 (1963).
6. This work was supported by NSF grant GA-1252 and by ONR contract Nonr 1286(10). We are grateful to D. R. Kester and C. Culberson for their helpful comments.

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Cytokinin from Soluble RNA of Escherichia coli: 6-(3-Methyl-2-butenylamino)-2-methylthio-9-β-D-ribofuranosylpurine

Abstract. *We have isolated a compound responsible for the cytokinin activity of soluble RNA from* Escherichia coli. *The structure, indicated as 6-(3-methyl-2-butenylamino)-2-methylthio-9-β-*D*-ribofuranosylpurine, $C_{16}H_{23}N_5O_4S$, on the basis of low- and high-resolution mass spectrometry, was established by unequivocal synthesis. The mass spectra, chromatographic behavior, and ultraviolet spectra of the compounds from natural and synthetic sources were identical.*

Acid hydrolyzates of *Escherichia coli* tRNA (*1*) were found to exhibit cytokinin activity in the tobacco callus bioassay (*2*), but since 6-(3-methyl-2-butenylamino)-9-β-D-ribofuranosylpurine (2iPA), the cytokinin-active minor riboside of several tRNA's (*3*), was not detectable in the enzymic hydrolyzates of *E. coli* tRNA (*4*), it was of special interest to isolate and identify the compound or compounds responsible for this activity. The approach, then, was to locate the component by following its route through the isolation procedure by means of the tobacco bioassay (*5, 6*) of aliquots.

Thirty grams of partially degraded *E. coli* B sRNA (*7*), containing 9.7 $O.D._{260}$ units per milligram, were hydrolyzed to nucleosides with lyophilized snake venom (*Crotalus adamanteus*, Sigma) and alkaline phosphatase (*8*). The hydrolysis procedure was similar to that of Hall (*9*). The RNA was dialyzed for 24 hours at 4°C before hydrolysis.

The nucleoside mixture was fractionated by partition chromatography on Celite-545 columns (*9*); the upper phase of a mixture of ethyl acetate, methoxyethanol, and water (4:1:2) was used for elution. The elution profile was similar to that reported by Hall (*10*) for yeast sRNA. Cytidine and guanosine were eluted together by washing the column with distilled water.

A small $O.D._{260}$ peak (fraction 1) emerging with the solvent front was highly active in the tobacco bioassay for cytokinins (*2*). The activity in fraction 1 was increased by acid hydrolysis (0.1*N* HCl, 100°C, 45 minutes) before bioassay. Under these conditions, activity could also be detected in the methyladenosine peak and, very weakly, in the adenosine peak. The uridine peak and water eluate were inactive, as were all fractions eluted from a control column to which a sample of the enzyme solution, incubated in the absence of RNA, was applied.

Thin-layer chromatography of samples of fraction 1 in Cellulose MN 300 F_{254} plates, with distilled water as a solvent, revealed four ultraviolet-absorbing spots. Cytokinin activity was associated with two poorly resolved spots moving at R_F 0.06 and 0.12 (compounds 1′ and 2′). Two fast-moving spots (compounds 3′ and 4′, R_F 0.64 and 0.82, respectively) were inactive in the tobacco bioassay. Compounds 1′ and 2′ could be resolved by thin-layer chromatography in 10 percent ethanol (R_F 0.10 and 0.31, respectively). Only compound 2′ exhibited cytokinin activity.

Compound 2′ was isolated from fraction 1 (see above) by chromatography on Whatman No. 1 paper. The composition of eluates from the chromatograms was monitored by bioassay and thin-layer chromatography. Chromatography of fraction 1, with distilled water as a solvent, separated the fast-moving compounds 3′ and 4′ from the cytokinin activity which was streaked from the origin to about R_F 0.4. Compound 2′ and a large amount of fluorescent material were present in the eluate from R_F 0.06 to 0.40. The chromatographic properties of the fluorescent substance were very similar to those of compound 2′. As a result, it was convenient to isolate the latter compound from the eluate of the region below R_F 0.06. The solids from this eluate were extracted twice with 1.5 ml volumes of chloroform-petroleum ether (2:1). The supernatant from this extraction contained considerable colored material together with most of compound 1′ and a small amount of compound 2′. The residue, consisting primarily of compound 2′, was chromatographed in 50 percent ethanol. One ultraviolet-absorbing band (R_F 0.85) was observed. The eluate from this band was rechromatographed in 20 percent ethanol and gave a single ultraviolet-absorbing band at R_F 0.58, which was again rechromatographed in 20 percent ethanol. Elution of the ultraviolet-absorbing band (R_F 0.58) yielded about 1 mg of white solids containing compound 2′ and a small amount of fluorescent material. This sample was used for determination of the structure of compound 2′.

The low-resolution mass spectrum of the active compound showed a fragmentation pattern parallel to that of