Osmotic Properties of Aqueous Solutions Containing Nucleosides

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Abstract □ The thermoelectric osmometer described in earlier publications was used to study the osmotic properties of 2′-deoxyadenosine, 2′-deoxyguanosine, uridine, cytidine, and thymidine in aqueous solutions ranging from 0.01 to 0.2 molal. Changes in resistance of a thermistor, ΔR, represent colligative properties which were plotted against corresponding molal concentrations. All points fell on intersecting straight lines, the points of intersection representing critical concentrations which are analogous to the critical concentrations reported previously for caffeine. This critical concentration is shifted to higher values by the presence of a fixed concentration of another nucleoside. Osmotic coefficients and complexing capacity numbers may be calculated by the methods used for caffeine data.

Keyphrases □ Osmotic properties—aqueous nucleoside solutions, calculation of osmotic coefficients and complexing capacity numbers □ Nucleosides, aqueous solutions—determination of osmotic properties, calculation of osmotic coefficients and complexing capacity numbers □ Complexing capacity numbers—determined for nucleosides in aqueous solutions □ Thermoelectric osmometry—osmotic properties of nucleosides in aqueous solutions

Because of the biological importance of nucleosides, it was decided to extend the thermoelectric osmometry reported for caffeine (1) to include certain nucleosides. Dilute solutions of cytidine, 2′-deoxyadenosine, 2′-deoxyguanosine, thymidine, and uridine in the concentration range between 0.01 and 0.2 molal were studied at 25° separately in pure water and also in dilute solutions of other members of the group.

This paper is concerned primarily with the presentation of experimental data which, like the caffeine data, can be given in terms of the slopes, intercepts, and intersections of straight lines representing the colligative property, ΔR, plotted against molal concentration. All measured points fall on a line within the limit of experimental accuracy. This treatment expedites usual calculations and is essential to show clearly the shift in point of intersection used to calculate complexing capacity numbers similar to those shown for caffeine.

EXPERIMENTAL

Instrumentation—The osmometer used in this work was described previously (1–4). It is essentially an ebulliometer in that condensing vapor elevates the temperature of a sample suspended in air which is saturated with solvent vapor. Solvent is held against the inner wall of the containing cell immersed in a water bath held at constant temperature by means of an air bath and an auxiliary heater. Slow temperature drifts of the order of 0.001° are compensated for by a reference thermistor in a different cell in the water bath.

New circuits developed after this work was completed provide greater convenience and economy (4). However, all of the other essential improvements described were used, but with greater need for manual control of temperature. The older bridge circuit was used throughout without loss of final accuracy. Absolute resistance values are of secondary importance because ΔR is the difference between the resistance of the measuring thermistor when in contact with pure solvent and when in contact with the sample under study. Both solvent and sample are suspended on the same thermistor in the same way.

Table I—Data Falling on Two Intersecting Straight Lines

Variable	Vehicle	Intercept[a], ohms	First Slope, $\Delta R/\Delta M$	Intersection	Second Slope, $\Delta R/\Delta M$
2′-Deoxyadenosine	Water	0.04	927.2	0.0152	597.4
2′-Deoxyadenosine	Thymidine, 0.02 molal	19.16	691.8	0.0360	330.0
2′-Deoxyadenosine	Uridine, 0.02 molal	18.36	767.0	0.0358	378.0
2′-Deoxyadenosine	2′-Deoxyguanosine, 0.01 molal	9.14	672.0	0.0565	342.0
2′-Deoxyguanosine	Water	0.03	925.0	0.0123	565.0
2′-Deoxyguanosine	Cytidine, 0.003 molal	2.72	897.0	0.0155	384.6
2′Deoxyguanosine	2′-Deoxyadenosine, 0.003 molal	2.98	833.8	0.0213	400.0
Uridine	Water	0.02	922.5	0.0568	789.4
Uridine	2′-Deoxyadenosine, 0.015 molal	13.83	799.8	0.1900	651.0
Uridine	2′-Deoxyguanosine, 0.012 molal	10.87	846.4	0.1631	659.5
Cytidine	Water	0.03	923.5	0.0412	823.5
Cytidine	Thymidine, 0.02 molal	18.29	846.4	0.1212	683.5
Cytidine	2′-Deoxyadenosine, 0.015 molal	13.98	805.0	0.1307	657.0
Cytidine	2′-Deoxyguanosine, 0.012 molal	11.08	834.5	0.1285	694.4
Thymidine	Water	0.04	924.4	0.0563	747.1
Thymidine	2′-Deoxyguanosine, 0.012 molal	11.10	811.2	0.1369	620.9
Thymidine	Cytidine, 0.02 molal	18.43	845.6	0.1154	660.0
Thymidine	2′-Deoxyadenosine, 0.015 molal	13.84	783.0	0.1458	604.5

[a] The intercepts are not experimental points.

Solutions—Solutions were made with distilled water on a molal basis. No further purification was carried out on the solid chemicals obtained from commercial sources[1]. However, an assay for percentage of water was made in each case by drying a small sample to constant weight over phosphorus pentoxide in a vacuum drying apparatus at the boiling point of absolute alcohol. The percentage of water found was used to correct the weight of solid used in preparing the various solutions.

As a test of purity, the following molecular weights were calculated by Eq. 4 from the osmotic data given in Table I and compared

[1] Solid chemicals were obtained from Nutritional Biochemicals except for 2′-deoxyadenosine which was obtained from General Biochemicals.

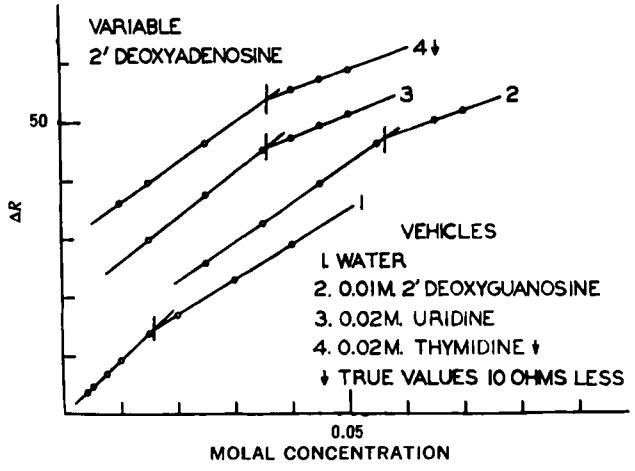

Figure 1—ΔR *in ohms* versus *molal concentration of 2'-deoxyadenosine. Arrow indicates displacement by 10 ohms to avoid crowding.*

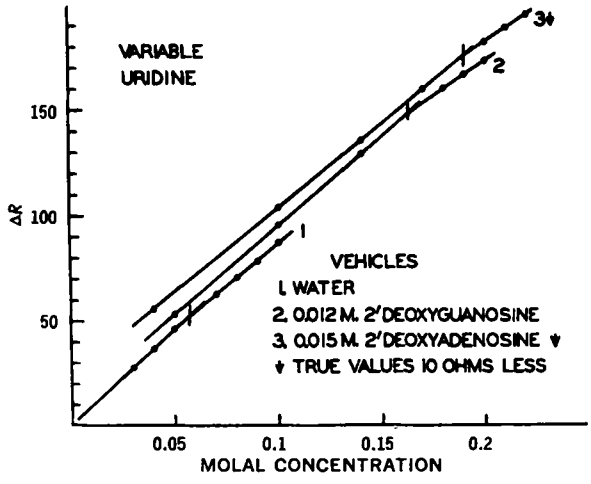

Figure 3—ΔR *in ohms* versus *molal concentration of uridine. Arrow indicates displacement by 10 ohms to avoid crowding.*

with the established formula weights (given in parentheses): 2'-deoxyadenosine (251.2), found: 250.3; 2'-deoxyguanosine (267.2), found: 266.8; cytidine (243.2), found: 243.3; thymidine (242.2), found: 242.0; and uridine (244.2), found: 244.5. Reagent grade (ASC Code 2376) B&A sucrose crystals were used to prepare the solutions used as reference standards.

Standardization—Aqueous solutions of sucrose at 0.01, 0.03, and 0.05 molal were found to have ΔR values of 9.3, 27.5, and 46.2 ohms, respectively. These three values may be treated by the method of least squares to give a slope of 922.3 ohms molal^{-1}. When the experimental values of 93.3 and 187.8 ohms for 0.1 and 0.2 molal, respectively, are corrected by dividing by the corresponding osmotic coefficients given by Robinson and Stokes (5) and included with the above values for the lower concentrations, a slope of 923.7 ohms molal^{-1} is found. This slope was used with all nucleoside data and should not be confused with values given in other papers. Large differences in standardization represent different thermistors from the same shipment. Small differences close to estimated experimental error are caused by slight changes in the cell itself. It is important to restandardize after any change in the instrument.

RESULTS

The experimental data on the nucleosides are given in Figs. 1–5 and are summarized in Table I. The figures present a fair estimate of the accuracy of the measurements and the goodness of fit. Each ΔR value shown is the average of two or more measurements on separate samples of the same solution. The slopes and intercepts shown in Table I were obtained by the method of least squares and are reported to one more significant figure than is probably warranted by the accuracy of the experimental work. This treatment is justified because these values are used in computing molecular weights, osmotic coefficients, and points of intersection. Intercepts are included as a measure of internal consistency.

Table I also provides data needed to calculate osmotic coefficients, ϕ, assuming the validity of the equation:

$$\phi = \Delta R/\Delta R_0 \qquad \text{(Eq. 1)}$$

The following equations can be derived by the application of elementary analytic geometry:

$$\phi = S_1/S_0 \qquad \text{(Eq. 2)}$$

$$\phi = S_1C_1/S_0M + S_2(M - C_1)/S_0M \qquad \text{(Eq. 3)}$$

Equation 2 applies at molal concentrations below the critical concentration (intercept) designated C_1. Equation 3 applies above the critical concentration, where S_2 is the second slope and M is the molal concentration under consideration. In both equations, S_1 is the first slope and S_0 is the slope obtained by standardization with sucrose. In Eq. 1, ΔR_0 is S_0M, and ΔR represents the solution under investigation at the molal concentration, M.

Osmotic coefficients are calculated from Table I by the method already described and are compared with values reported by other workers (6–8). These results and comparisons are given in Table II for selected concentrations and also for the exact concentration

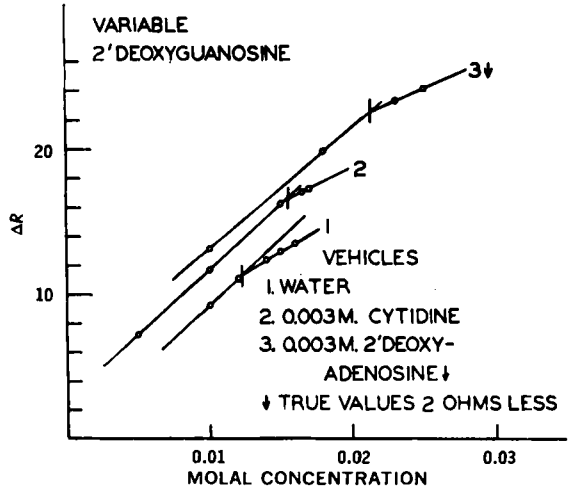

Figure 2—ΔR *in ohms* versus *molal concentration of 2'-deoxyguanosine. Arrow indicates displacement by 2 ohms to avoid crowding.*

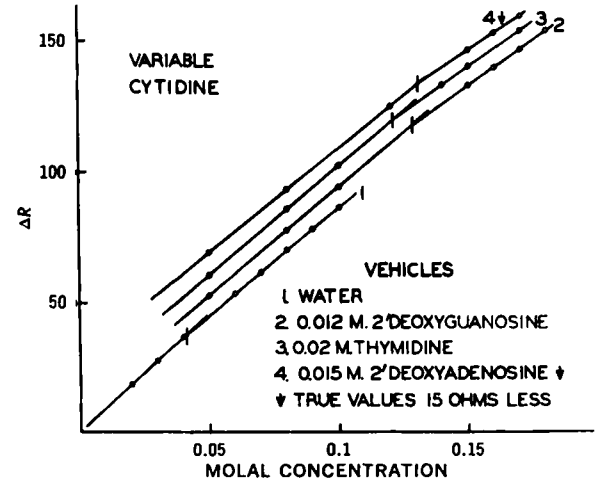

Figure 4—ΔR *in ohms* versus *molal concentration of cytidine. Arrow indicates displacement of 15 ohms to avoid crowding.*

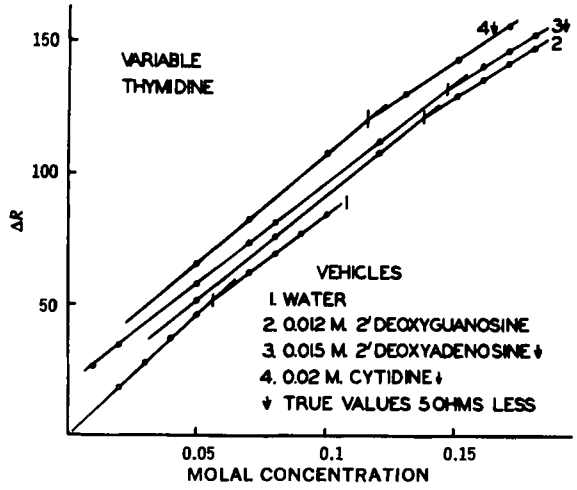

Figure 5—ΔR *in ohms* versus *molal concentration of thymidine. Arrow indicates displacement of 5 ohms to avoid crowding.*

specified by other workers. The earlier work involved curve fitting and was not primarily directed to this region of concentration.

DISCUSSION

Osmolal Concentrations and Molecular Weights—All ΔR values may be converted to osmolal concentration by dividing by the corresponding value for sucrose, which is conveniently expressed as $S_0M = \Delta R_0$, using the symbolism of Eqs. 1 and 3. Below the critical concentration, it may be assumed that dissociation is negligible and that osmolal and molal concentrations are the same. It is, therefore, possible to calculate molecular weights from ΔR values in this region. Assume that any arbitrary value, F, was used for the molecular weight of the solute in calculating a tentative molal concentration, T, from weight, F, of solute in 1 kg. of solvent. On this basis, $\Delta R = S_1T$, $\Delta R_0 = S_0T$, and the osmolal concentration, $\Delta R/\Delta R_0$, the true molal concentration in this region, becomes S_1/S_0 which is F/mol. wt. Therefore, molecular weight is given by the equation:

$$\text{mol. wt.} = F(S_0/S_1) \qquad \text{(Eq. 4)}$$

Equation 4 was used to calculate the molecular weights listed in connection with the preparation of solutions.

Complexing Capacity Numbers—Calculations based on the values given in Table I and the method outlined for caffeine (1) show that, just as for caffeine, the complexing capacity numbers approach integers. However, higher values predominate, especially for the complexing capacity numbers with uridine, cytidine, and thymidine where values of 9, 6, and 6, respectively, are found for 2'-deoxyadenosine and values of 9, 7, and 9, respectively, are found for 2'-deoxyguanosine.

Self-Association—The fact that a critical concentration is observed for all pure substances studied suggests self-association, possibly of the type $nA = A_n$ or $2A = A_2$, $A_2 + A = A_3$, *etc.* By writing appropriate equilibrium expressions, both molal and osmolal concentrations can be generated by assigning reasonable values to K and, for the first type, n also. For each choice, the monomer concentration (A) was varied systematically over a sufficient range to show that calculated osmolal concentrations plotted against calculated molal concentrations did indeed generate curves that approached intersecting straight lines for some values chosen for the parameters. The choice of $n = 4$ and $K = 100$ generates such a curve for equilibria of the first type, and the choice of $K_1 = 1$, $K_2 = 2$, and $K_3 = 4$ serves approximately for the multiple-equilibrium hypothesis. These calculations were greatly facilitated by the use of a computer[2].

It must not be assumed that the authors are offering evidence favoring specific equilibrium constants or models. It is quite possible that combinations occur that are not approximated by either hypothesis considered here. However, it is important to show that the presentation of accurate measurements by giving slopes and intersection is not inconsistent with more conventional treatment involving a consideration of complex equilibria.

[2] IBM 360 computer programmed by Mr. Phillip Johnson of the University of California San Francisco Information Service.

Table II—Comparison between Calculated Osmotic Coefficients (ϕ) and Values Reported in the Literature at 25°

Molal Concentration	ϕ Calc.	ϕ Lit.
	Deoxyadenosine in Water	
0.005	1.015	—
0.010	1.014	—
0.015	1.009	—
0.01725	0.965	0.84 ± 0.07[a]
0.020	0.921	—
0.025	0.867	0.900 ± 0.01[b]
0.030	0.830	—
0.0345	0.806	0.760 ± 0.04[a]
0.040	0.785	—
0.050	0.757	0.800 ± 0.005[b]
	2'-Deoxyguanosine in Water	
0.010	1.006	—
0.012	1.006	—
0.014	0.957	—
0.015	0.934	—
0.016	0.914	—
	Uridine in Water	
0.030	1.001	—
0.040	1.001	—
0.050	1.001	0.969[c]
0.060	0.993	—
0.070	0.973	—
0.080	0.958	—
0.094	0.943	0.952, 0.962[c]
0.095	0.942	0.933[c]
0.096	0.941	0.937[c]
0.100	0.938	0.943[c]
	Cytidine in Water	
0.030	1.002	—
0.040	1.002	—
0.050	0.983	0.967[c]
0.070	0.957	—
0.080	0.949	—
0.0941	0.941	0.941[c]
0.0948	0.940	0.939[c]
0.0955	0.940	0.936[c]
0.1000	0.938	0.935[c]
	Thymidine in Water	
0.040	1.003	—
0.050	1.003	—
0.070	0.965	—
0.090	0.931	—
0.100	0.918	0.905[b]

[a] From *Reference 8.* [b] From *Reference 7.* [c] From *Reference 6.*

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Chemical Constituents of the Gentianaceae V: Tetraoxygenated Xanthones of *Swertia chirata* Buch.-Ham.

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Abstract □ Nine tetraoxygenated xanthones [1,5,8-trihydroxy-3-methoxyxanthone (I), 1-hydroxy-3,5,8-trimethoxyxanthone (II), 1-hydroxy-3,7,8-trimethoxyxanthone (III), 1,8-dihydroxy-3,5-dimethoxyxanthone (IV), 1,8-dihydroxy-3,7-dimethoxyxanthone (V), 1,3,6,7-tetrahydroxyxanthone-C_2-β-D-glucoside (mangiferin, VI), 1,3,8-trihydroxy-5-methoxyxanthone (VII), 1,3,5,8-tetrahydroxyxanthone (VIII), and 1,3,7,8-tetrahydroxyxanthone (IX)] were isolated from the roots and aerial parts of *Swertia chirata* Buch.-Ham. (Gentianaceae) collected from Nepal and India. The identity of the xanthones was established by direct comparison with reference materials in most cases, preparation of derivatives, and spectral evidence (UV, IR, proton magnetic resonance, and mass spectrometry). Among these xanthones, II was not encountered before in nature and VI was found for the first time in the genus swertia. The biogenetic significance of the co-occurrence of several tetraoxygenated xanthones, of varying oxygenation patterns, in absence of the "standard" 1,3,5- and 1,3,7-trioxygenated xanthones in *S. chirata* and in related species is appraised. In addition to the tetraoxygenated xanthones (I–IX), a number of heterosides, triterpenes, and monoterpene alkaloids were isolated from this plant. Preliminary pharmacological screening of the total xanthones of *S. chirata* indicated that the medicinal properties ascribed to the plant extracts were due to these constituents.

Keyphrases □ *Swertia chirata* Buch.-Ham. (Gentianaceae)—isolation, identification of nine tetraoxygenated xanthones □ Xanthones, tetraoxygenated—isolation, identification from *Swertia chirata* Buch.-Ham. (Gentianaceae) □ Medicinal plants—isolation, identification of tetraoxygenated xanthones from *Swertia chirata* Buch.-Ham.

Swertia chirata Buch.-Ham. (Gentianaceae) is widely distributed in India in the temperate Himalayas between 4000 and 10,000 ft., from Kashmir to Bhutan, and in Khasia Hills between 4000 and 5000 ft. (1). It also grows abundantly in Nepal. The plant is well known for its uses in the Indian system of medicine for a variety of purposes (1, 2). The extract of the plant is used as a bitter stomachic, a febrifuge, an anthelmintic, a remedy for scanty urine, in epilepsy, and for certain types of mental disorders. The total annual requirement of this pharmacopeial drug in India is about 400 quintals (3).

Previous phytochemical investigation by Dalal and Shah (4) reported the presence of only one xanthone, 1,8-dihydroxy-3,5-dimethoxyxanthone, in the whole plant. But no attempt was made to determine the active principle of this vegetable drug. Interest in the detailed investigation of this plant was piqued for two reasons: (*a*) xanthone-bearing plants generally elaborate multiple xanthones (5), and (*b*) in *Calophyllum inophyllum* L. (Guttiferae), variations in the types of xanthonic constituents were recorded (6–8) due to ecological variations. In the present study, *S. chirata* plants[1] were collected from Nepal and India to test these possibilities.

EXPERIMENTAL[2]

The general procedure described under *Isolation of Xanthones from Aerial Parts of* S. chirata was followed for the isolation of xanthones from the roots. The other chemical constituents were isolated following a procedure shown in Scheme I.

Isolation of Xanthones from Aerial Parts of *S. chirata*—Dried and milled aerial parts of *S. chirata* (1 kg.) were continuously extracted (soxhlet) for 20 hr. with petroleum ether (60–80°), and the defatted plant material was subsequently extracted (20 hr.) with ethanol. The two extracts were separately processed.

Treatment of Petroleum Ether Extract—The petroleum ether extract was concentrated (about 250 ml.) under reduced pressure, and the concentrate was kept overnight at ordinary temperature when a yellow amorphous solid (Fraction A) separated. The solid was collected by filtration, and the mother liquor was evaporated to dryness (Fraction B).

Separation of Xanthones Present in Fraction A—Fraction A (1.8 g.) was dissolved in ether (500 ml.), and the phenolic and nonphenolic components were separated by extraction with aqueous

[1] The plant material of Indian origin was a gift from Dr. S. P. Wahi, Department of Pharmaceutics, Banaras Hindu University, and that from Nepal was supplied by Mr. R. A. Panjiar, Janakpur Dhum, Nepal. Voucher specimens have been preserved at the Department of Pharmaceutics, Banaras Hindu University, Varanasi-5, India.

[2] Melting points were determined on a Toshniwal melting-point apparatus, in open capillaries, and are uncorrected. UV spectra were recorded in a Cary 14 spectrophotometer in aldehyde-free ethanol (95%). IR spectra were recorded with Perkin-Elmer 237/257 instruments in KBr and mineral oil. Proton magnetic resonance (PMR) spectra were obtained on a Varian A-60 D spectrometer, using deuteriochloroform and dimethyl sulfoxide-d_6 as the solvents. Mass spectra were determined on an AEI MS-9 instrument operated at 70 ev. Combustion analyses were performed by the Central Drug Research Institute, Lucknow, India. Separation by column chromatography was carried out by using silica gel (60–120 mesh, British Drug Houses), and layer chromatographic experiments were conducted with silica gel G (E. Merck). Four solvent systems [benzene–acetic acid (60:1, Solvent 1), chloroform–benzene (5:2, Solvent 2), chloroform–benzene (1:1, Solvent 3), and *n*-butyl alcohol–acetic acid–water (4:1:2, Solvent 4)] were used as the developers, and iodine vapor was used for staining purposes.