Osmotic Properties of Aqueous Solutions Containing Caffeine

By FRANK M. GOYAN and HANNA N. BORAZAN

An improved form of the thermoelectric osmometer developed by one of the authors has been used to study solutions of *p*-aminobenzoic acid, citric acid, sodium citrate, sodium salicylate, sodium benzoate, morphine hydrochloride, and papaverine hydrochloride alone and in combination with caffeine. The measured colligative property is plotted against concentration of caffeine. These data are described by two intersecting straight lines for each substance. The point of intersection occurs at different positions on the caffeine scale depending upon the nature and concentration of the complexing agent. The shift along the caffeine scale from the break in the pure caffeine data is divided by the concentration of the complexing agent to give a characteristic complexing capacity number. Osmotic coefficients may be calculated from tabulated slopes.

THE IDEA that the molecular forces related to the solvent effect of caffeine may be involved in attachments of biological significance was suggested by Weil-Malherbe (1). Higuchi and his colleagues (2–6) have established a relationship between the solubilizing action of caffeine and its tendency to form complexes and aggregates. The combination of these two ideas suggests that the instrumentation developed by Johnson and Goyan (7–9) may be adapted to the study of properties related to drug action.

A preliminary survey of the problem indicated the need for greater sensitivity and temperature stability. These improvements were approached on a developmental basis while measurements were in progress. The final outcome was an approximately 10-fold increase in sensitivity with considerable improvement in stability and reproducibility, compared to the same instrument before modification.

EXPERIMENTAL

The circuit and technique previously described (7–9) were not changed except that the regulated power supply (Leeds and Northrup part No. 099012) was installed in place of the battery and calibrating circuit of the Speedomax recorder. The slide wire of the recorder was connected to this power source through two 400-ohm resistors which served as end coils. A 100-ohm resistor was connected across the regulated power supply. The extemporaneous arrangement of 200-ohm precision resistors increased the sensitivity of the recorder from 10 mv. full scale to approximately 1 mv. full scale. Because of the fact that the recorder is used as a nul instrument and calibrated against the resistance box, this temporary arrangement was used throughout the course of the work. It is planned to install the recommended range card for future work.

Attempts to stabilize the temperature regulation and otherwise to improve the stability and reproducibility of the instrument continued throughout the course of the measurements. Manual adjustment and empirical uniformity of method had to be resorted to in the early phases. The following improvements are significant: (*a*) a 4-L. Dewar flask was substituted for the original 1-L. flask used to maintain water at constant temperature. (*b*) The Dewar flask was provided with an insulated metal cover to which was attached brass holders to keep the cells in a vertical position under the water. (*c*) The part of the cell extending above the cover was protected from draft by means of a plastic cylinder which was split to allow half to be removed temporarily while adding sample. (*d*) A thin glass rod less than 1 mm. in diameter was attached to the bottom of the platinum coil around the thermistor. This had the effect of maintaining nearly equal drops by drawing away excess sample. The rod extended to the lowest level of the platinum gauze. (*e*) Cells not adequately vented seemed to cause trouble. This was corrected in one case by sealing a glass tube just below the platinum gauze and bringing the open end of this tube up to the level of the top of the sample tube. (*f*) Temperature control was improved by the control of the air bath with a modified Melabs proportional temperature controller, using a thermistor sensing element. No attempt was made to heat or cool the water, except for initial manual adjustment. The stirring motor was operated from a constant voltage source. Once the correct differential between air and water was established, temperature drift was too slow to influence the readings when using the reference thermistor in the circuit. It was found necessary to improve the insulation of the air bath and to manipulate the droppers through holes draped with cloth.

Solutions were made on a molal basis using ordinary distilled water. The solid needed to make the most concentrated solution of a series was weighed out and the other concentrations made on a molal basis by dilution with the appropriate vehicle. Because of the concurrent study of the instrument it was necessary to discard preliminary results in many cases. However, all of the data included in this paper were taken after proper adjustments were made. All points fall within the circles shown on the figures. USP or equivalent quality chemicals obtained from reliable sources were used without further purification.

RESULTS

All results obtained were plotted with ΔR as ordinate and molal concentration as abscissa. It was found that intersecting straight lines could be used to express all data given in Table I within the limit of experimental error. These intersecting straight lines are of special interest because it was discovered that the point of intersection shifted as a function of the nature and concentration of the

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TABLE I—DATA FALLING ON TWO INTERSECTING STRAIGHT LINES

Variable	Vehicle	Intercept, ohms	First Slope dR/dM	Intersection M	Second Slope dR/dM
Caffeine	Citric acid, 0.1 M	108.9	835	0.0250	553
Caffeine	Morphine HCl, 0.03 M	52.3	658	0.0825	320
Caffeine	PABA, 0.005 M	4.2	743	0.0276	528
Caffeine	PABA, 0.010 M	8.2	659	0.0331	498
Caffeine	PABA, 0.015 M	12.7	605	0.0365	445
Caffeine	Papaverine HCl, 0.03 M	39.4	458	0.0832	130
Caffeine	Sodium benzoate, 0.06 M	106.8	554	0.0807	325
Caffeine	Sodium citrate 0.01 M	29.8	880	0.0214	554
Caffeine	Sodium salicylate, 0.02 M	35.3	579	0.0808	330
Caffeine	Water	0.0	880	0.0232	558
Morphine HCl	Water	0.0	1760	0.0267	1481
Papaverine HCl	Water	0.0	1760	0.0120	1034

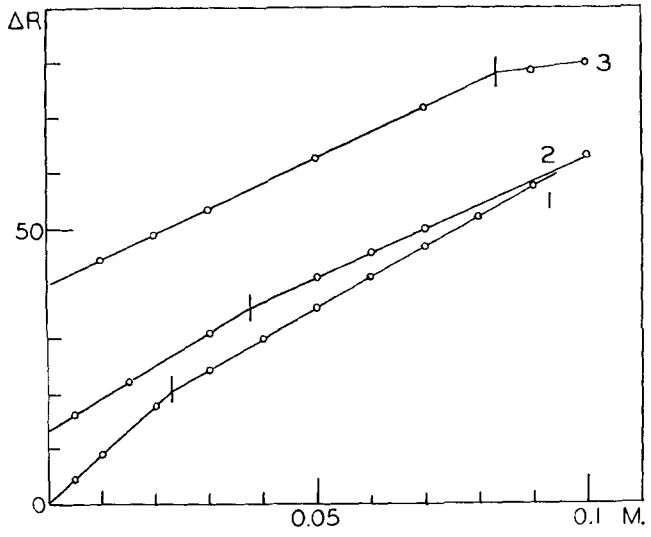

Fig. 1—Plot of measured differences in resistance in ohms plotted against molal concentration of caffeine in the following vehicles: 1, water; 2, 0.015 M *PABA; 3, 0.03* M *papaverine hydrochloride. The intersection of two straight lines is marked by a short vertical line.*

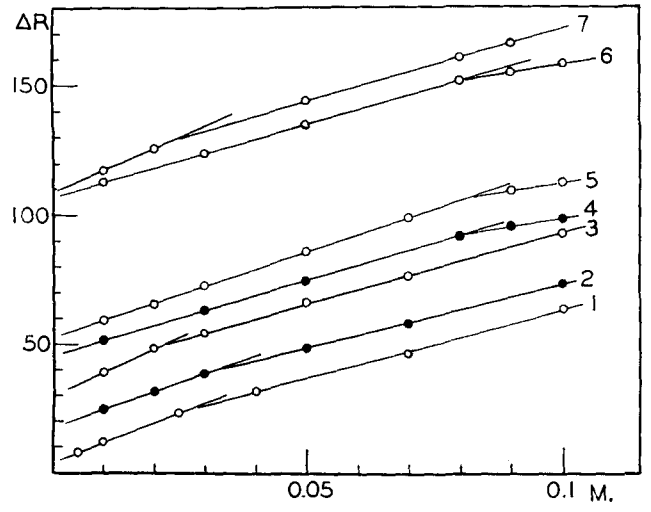

Fig. 2—Similar to Fig. 1 except that points represented by solid disks were raised 10 ohms in order to avoid crowding. Numbers refer to the following vehicles: 1, 0.005 M *PABA; 2, 0.01* M *PABA; 3, 0.01* M *sodium citrate; 4, 0.02* M *sodium salicylate; 5, 0.03* M *morphine hydrochloride; 6, 0.06* M *sodium benzoate; 7, 0.1* M *citric acid.*

compound used to prepare the vehicle. For these reasons, all slopes and intercepts shown graphically were checked by the method of least squares.

The same data are displayed in Figs. 1 and 2 to show the shift in the point of intersection of the two lines for caffeine. This difference between the intersection in water and in the solution under study is divided by the concentration of the vehicle solute to yield the complexing capacity number shown in Table II. It is difficult to estimate the accuracy of these values, but it is almost certain that the last figure shown in the column of calculated values is not significant. The close approach to simple integers suggests that these integers are in

TABLE II—COMPLEXING CAPACITY NUMBERS WITH CAFFEINE

Vehicle	Calculated from Table I	Nearest Integer	Increased Solubility Ref.
Citric acid, 0.1 *M*	0.02	0	(10)
Morphine HCl, 0.03 *M*	1.98	2	...
PABA			
0.005 *M*	0.88	1	(4, 5)
0.010 *M*	0.99		
0.015 *M*	0.89		
Papaverine HCl, 0.03 *M*	2.00	2	...
Sodium benzoate, 0.06 *M*	0.96	1	(2, 10)
Sodium citrate, 0.01 *M*	−0.18	0	...
Sodium salicylate, 0.02 *M*	2.88	3	(3, 10)

necessary to state complexing capacity numbers as integers. It is quite possible that the decimal fractions given in the first column of Table II have some significance. The important conclusion is that these numbers do exist and can be measured.

It is a well-known fact that a true colligative property of sucrose plotted against concentration does not fall exactly on a straight line (11). The treatment of the present data was indicated by interest in the complexing numbers and by the fact that the authors are not yet in a position to state absolute values based upon empirical corrections. However, all of the slopes given may be used to calculate apparent osmotic coefficients for pure substances at a concentration of 0.1 *M* by the equation:

$$\phi = \frac{S_1 C_1 + S_2(0.1 - C_1)}{88} \qquad \text{(Eq. 1)}$$

where ϕ is the osmotic coefficient, S_1 is the first slope, C_1 is the concentration at the intersection, and S_2 is the second slope of Table I. Similar equations may be derived for other concentrations. Comparison of the present work with the work of others shows some discrepancies not easily explained. Blake and Harris (12), working near the freezing point of water, have assumed that caffeine behaves as an ideal solute. Several papers combined (13–15) indicate that the osmotic coefficient of caffeine is about 0.65 as compared to a value of 0.72 calculated by Eq. 1.

TABLE III—DATA FALLING ON SINGLE STRAIGHT LINES

Variable	Vehicle	Intercept, ohms	Slope dR/dM	Last Point *M*	Number of Points
Benzoic acid	Water	0.0	880	0.025	5
Citric acid	Water	2.3	1000	0.20	6
Citric acid	Caffeine 0.01 *M*	8.8	1012	0.10	5
Citric acid	Caffeine 0.1 *M*	61.5	1075	0.10	4
PABA	Water	0.0	873	0.03	5
Salicylic acid	Water	0.0	880	0.015	3
Sodium benzoate	Water	0.0	1760	0.05	3
Sodium citrate	Water	4.2	2621	0.10	4
Sodium citrate	Caffeine 0.02 *M*	21.6	2629	0.10	4
Sodium salicylate	Water	0.0	1760	0.10	4
Sucrose	Water	0.1	882	0.20	6

some way related to the stoichiometry of complex formation.

A few other systems were studied in the hope that they might throw some light on the meaning of these complexing capacity numbers. These data are shown in Table III. The straight lines represented by slope and intercept pass through all of the measured points within the limit of experimental error. The intercepts are not experimental points. The experimental value for zero concentration is always zero because of the practice of using a drop of pure water to establish a base resistance reading before substituting drops of solution. In every case a 0.01 *M* or lower concentration was included in the series.

DISCUSSION OF RESULTS

The discovery of caffeine complexing numbers reported in this paper present new information about a few compounds. Emery and Wright (10) report that citric acid is a more effective solubilizing agent for caffeine than is sulfuric acid. The value of zero for the caffeine complexing number for citric acid and for sodium citrate shown in Table II indicates that the interaction with citric acid implied by the experiments of Emery and Wright is different from interaction with compounds of Table II known to have specific biological activity. It is not

The present work adds a new dimension to the study of caffeine complexes and is in agreement with the work of Donbrow and Jan (16) concerning the absence of aggregates in solutions of caffeine more dilute than 0.02 *M*. However, something like a critical micelle concentration may be found at 0.023 *M*. The complexing capacity number of unity for sodium benzoate may be regarded as being in agreement with Donbrow and Jan. It is interesting to speculate that the higher complexing capacity numbers found for morphine, papaverine, and sodium salicylate are in some way related to biological activity.

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Osmotic coefficient—calculated, equation
Thermoelectric osmometer—improved

Triterpenic Constituents of *Lepechinia chamaedryoides*

By MARIO SILVA

Betulinic acid was isolated and identified from *Lepechinia chamaedryoides* collected in the summer, and ursolic acid was found in plant material collected in the spring.

During a current project dealing with Chilean flora (1) the author had occasion to reexamine *Lepechinia chamaedryoides* in order to study the compound isolated by Alvarez (2), and to see how constant it is in this plant during the year.

The petroleum ether-soluble fraction of plant material collected during March (summer) yielded betulinic acid (3) characterized through its derivatives. Betulinic acid was also isolated from the benzene-soluble fraction. In this plant material no other triterpenic acid could be detected.

From plant material collected in November (spring), ursolic acid (4) was obtained which gave a methyl ester with a rather peculiar melting point (105°). Therefore, it was characterized through several derivatives. Betulinic acid could be detected by thin-layer chromatography only as its methyl ester.

These different results appear to be a seasonal variation of the triterpenic constituents.

EXPERIMENTAL[1]

Isolation of Betulinic Acid—Stems and leaves of *Lepechinia chamaedryoides* collected in March near Concepción, were dried at 80° with air circulation, and 2100 Gm. of the dried and ground plant was extracted in a Soxhlet apparatus with petroleum ether (b.p. 65–75°) to exhaustion. This solution was concentrated to yield 109 Gm. of a dark green product. This product was crystallized several times from ethanol to yield 11 Gm. of a crude acid m.p. 286°.

Betulinic Acid Methyl Ester—This ester was prepared by diazomethane treatment in ether solution; crystallized from ethanol as colorless crystals with m.p. 221°, $[\alpha]_D$ +4.4° (chloroform, c 0.44), $\nu_{max.}^{Nujol}$ 3610, 1695, and 1637 cm.$^{-1}$, $\nu_{max.}^{CHCl_3}$ 1724, 1647 cm.$^{-1}$.

Anal.—Calcd. for $C_{31}H_{50}O_3$: C, 79.10; H, 10.71. Found: C, 79.34; H, 10.69.

Betulin Diacetate—The betulinic acid methyl ester on $LiAlH_4$ reduction using ether as solvent gave an noncarbonilic compound which on pyridine–acetic anhydride acetylation at room temperature gave a diacetate (5), m.p. 222°, $[\alpha]_D$ + 28.7° (chloroform, c 0.48), $\nu_{max.}^{Nujol}$ 1732, 1640, and 1250 cm.$^{-1}$, τ = 7.9 (two acetates).

Anal.—Calcd. for $C_{34}H_{54}O_4$: C, 77.52; H, 10.33. Found: C, 77.73; H, 10.53.

The betulinic acid obtained was further characterized through several derivatives.

Benzene Extract—The defatted plant material was dried and the benzene-soluble constituents were extracted. This solution was concentrated to yield 27 Gm. of product. This product through crystallizations, first from petroleum ether and later with ethanol, gave in a good yield an acid, m.p. 285–286° identical with the betulinic acid previously isolated.

Isolation of Ursolic Acid—Stems and leaves of *Lepechinia chamaedryoides*, collected in November (spring) near Concepción, were dried at 80° with air circulation, and 4611 Gm. of the dried and ground plant was extracted in a Soxhlet apparatus with benzene to exhaustion. This solution was concentrated to yield 253 Gm. of a dark green product, and 150 Gm. of this product was chromatographed over alumina grade III to give crude ursolic acid m.p. 250°, $[\alpha]_D$ + 52.8° (pyridine, c 0.35).

Ursolic Acid Acetate—Treatment of the acid with pyridine–acetic anhydride gave an acetate, m.p. 248°,

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[1] Melting points (uncorrected) were performed on a Kofler block. Rotations were measured at 20°. The microanalyses were performed by Dr. Alfred Bernhardt's Institute, Mülheim, Germany, and at the Chemistry Department, Imperial College, London, England. Ultraviolet spectra were recorded in solution in absolute ethanol on a SP 700 spectrophotometer. Infrared spectra were recorded on a Perkin-Elmer 137 spectrophotometer. Nuclear magnetic resonance spectrum was determined on a Varian A-60 spectrometer using deuterochloroform as solvent and tetramethylsilane as an internal reference.