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COOPERATIVE BINDING OF POTASSIUM IONS TO *TRANS*-ISOHUMULONE IN THE PRESENCE OF DIVALENT AND TRIVALENT CATIONS

William J. Simpson* & Paul S. Hughes
BRF International, Nutfield, Surrey, RH1 4HY, UK

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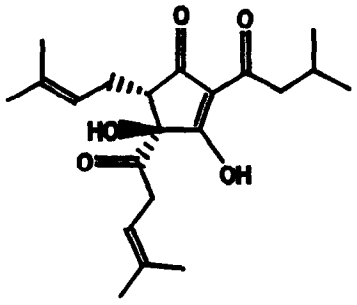
Abstract: In aqueous solution, the hop bitter acid *trans*-isohumulone (0.1 - 1.0 mM) was unable to bind K^+ (0.1 mM). In the presence of divalent cations, such as Mn^{2+}, Mg^{2+}, Ni^{2+} and Ca^{2+}, and trivalent cations, such as La^{3+} and Al^{3+}, it bound K^+ with an affinity which depended on the identity and concentration of the polyvalent cation.

Trans-isohumulone (1), derived from the flowers of the hop plant *Humulus lupulus* L., is one of the major bitter components of beer[1]. It also has antibacterial properties, acting as an ionophore of the mobile-carrier type. It inhibits growth of Gram-positive bacteria by exchanging H^+ for cellular divalent cations, such as Mn^{2+}. Monovalent cations stimulate the antibacterial activity of (1)[2]. In experiments with resting cell suspensions (1) was devoid of protonophoric activity until a monovalent cation such as K^+, Na^+ or Rb^+ was added to the extracellular medium[3]. These effects may be due either to an influence of the monovalent cations on the bacterial cells or to their interaction with (1).



(1)

Hop bitter acids such as (1) also stabilize beer foams and promote foam cling by interacting with a range of hydrophobic peptides present in beer[4]. A synergistic effect of divalent cations (such as Ni^{2+}) and hop bitter acids with respect to their ability to enhance the stability of beer foams has been

previously reported[5] but not adequately explained[6]. The results presented here provide an insight into the nature of both the ionophoric properties of (1) and its ability to stabilize protein foams in the presence of divalent cations.

Divalent and trivalent cations induce changes in the UV spectrum of (1) in aqueous solution and, more readily, in methanolic solution[7]. Monovalent cations do not change the UV spectrum of (1) even when present at a 300-fold molar excess. Using an ion-selective electrode of the liquid membrane type (Orion Research UK) we have found the K^+ activity of a 0.1 mM solution of KCl in 0.11 M sodium 3,3'-dimethylglutarate buffer[8] (pH 4.00) to be independent of the concentration of (1) over the range 0 - 0.8 mM at 25°C. In the presence of Mn^{2+}, the K^+ activity was reduced by (1) in a concentration-dependent fashion (Figure 1). Similar effects were observed when Mn^{2+} was replaced by Mg^{2+}, Ni^{2+}, Ca^{2+}, La^{3+} or Al^{3+}. When 0.11 M lithium 3,3'-dimethylglutarate buffer (pH 4.00) was used as the test medium[8] we obtained similar results, indicating that neither Li^+ nor Na^+ compete with K^+ for binding to (1) to any great extent. Control experiments showed that the presence of (1) or its metal complexes did not affect the electrochemical response of the K^+-selective/reference electrode combination to K^+. The degree to which K^+ was chelated was also dependent on the concentration of divalent or trivalent cation present.

The binding of K^+ to (1) was reversible and dependent on the activity of the divalent or trivalent cation, since addition of disodium ethylenediaminetetraacetic acid (EDTA) to the complex formed between K^+, Mn^{2+} and (1) resulted in the K^+ being released, as indicated by the return of the electrode response to its original value within 5 - 10 sec of addition of EDTA. At high concentrations of (1) in the presence of Al^{3+} some of the complex was precipitated from solution leading to a partial restoration of the original K^+ activity.

UV spectra of (1) recorded in the presence of KCl, $MnCl_2$ or both KCl and $MnCl_2$ showed that the spectral changes induced by Mn^{2+} were neither enhanced nor reduced by K^+ and confirmed that K^+ did not affect the spectrum of (1). This indicates that Mn^{2+} binds to the major chromophore of (1), the β-triketone group, while K^+ binds to a group which has little influence on the absorption spectrum. It is probable that the interactions between (1) and each of the cations involve ion-dipole attractions. This hypothesis is supported by the finding that binding of divalent cations to (1), as assessed by spectrophotometry, is independent of the ionization state of (1)[9]. In addition, no protons are released from, or taken up by (1) when K^+, Mn^{2+} or both ions are added to either its ionized or undissociated forms in aqueous solution[10].

Cooperative binding of K^+ in the presence of divalent cations is also seen with the hop compound (-)-humulone, but not with its hydrolysis product *trans*-humulinic acid or with the chelating agent EDTA (results not shown), indicating that the observed effects are unlikely to be experimental artefacts.

These findings suggest that the stimulatory effect of monovalent cations on the antibacterial activity of *trans*-isohumulone may be due to their effect on the physicochemical properties of the hop bitter acid. Thus, in the presence of K^+, the ability of (1) to transport Mn^{2+} and H^+ across the cell membranes of bacteria may be enhanced.

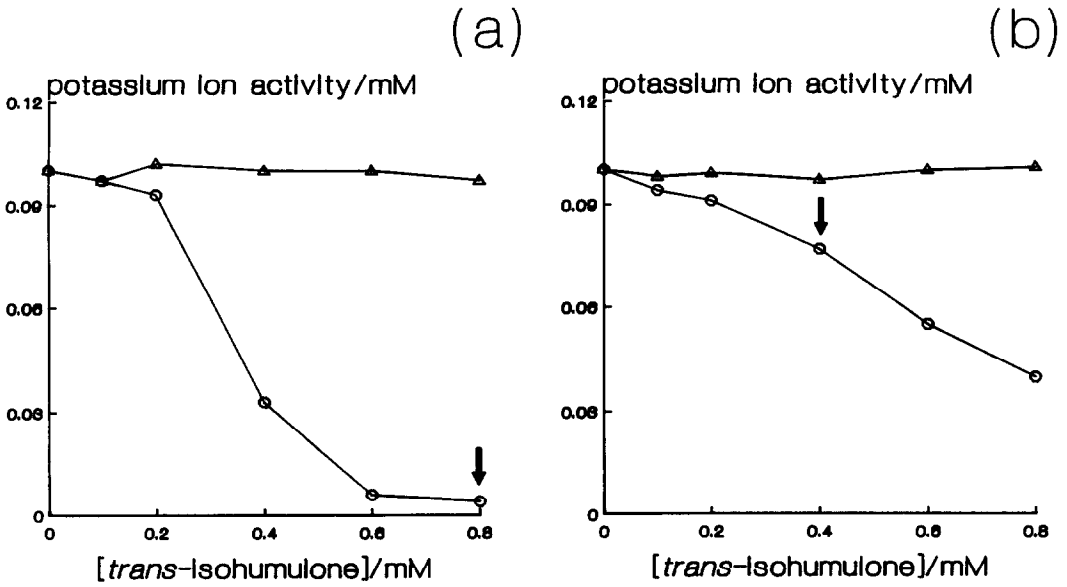


Figure 1. Cooperative binding of K^+ to *trans*-isohumulone in the presence of divalent cations. KCl (0.1 mM) was added to 0.11 M sodium 3,3'-dimethylglutarate buffer (pH 4.00) and the K^+ activity measured at 25°C in the presence (O) and absence (Δ) of (a) 0.74 mM Mn^{2+} or (b) 0.74 mM Ni^{2+}. Similar results were obtained with Mg^{2+}, Ca^{2+}, Al^{3+} and La^{3+}. Arrow indicates the onset of precipitation.

The ability of divalent cations to enhance the ability of hop bitter acids to stabilize beer foams could be explained by assuming that binding of compounds such as (1) to the charged amino groups on foam active peptides *via* ion-dipole interactions may be enhanced by simultaneous binding of a divalent cation to the hop bitter acid[11]. We have not been able to test this hypothesis directly as suitable methods for measurement of ammonium ion and peptide ion activity are not avaliable.

References and Notes

1. For detailed reviews on the chemistry of hop compounds and hop-derived compounds see (a) Stevens, R. *Chem. Rev.* **1967**, *61*, 19.; (b) Verzele, M. *J. Inst. Brew.* **1986**, *92*, 32.
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5. Rudin, A.D. *J. Inst. Brew.*, **1958**, *64*, 238.
6. (a) Asano, K.; Hashimoto, N. *Rep. Res. Lab. Kirin. Brew.*, **1976**, *19*, 9; (b) Roberts, R.T. *J. Inst. Brew.*, **1976**, *82*, 282.
7. *Trans*-isohumulone was prepared by photoisomerization of (-)-humulone as described by Clarke, B.J.; Hildebrand, R.P. *J. Inst. Brew.* **1965**, *71*, 26. All criteria of identity and purity were satisfactory. Absorption spectra were recorded over the range 200 - 400 nm in quartz cells of 1 cm path length at 20°C. The effects of divalent and trivalent cations on the electronic spectra of (1) will be reported in detail elsewhere.
8. The solution was prepared by adding 0.1 M NaCl to 0.01 M sodium 3,3'-dimethylglutarate buffer. In some cases, LiCl was added to lithium 3,3'-dimethylglutarate buffer. The internal filling solution of the calomel reference electrode was selected to match the test medium in each case.
9. The equilibrium pKa value of (1) is 3.1 (ref. 2); the interaction of (1) with divalent cations was not affected by changes in pH in this region.
10. The binding of ligands to weak acid chelating agents often results in release of H^+. See Albert, A.; Serjeant, E.P. The Determination of Ionization Constants: A Laboratory Manual. Chapman and Hall: London, 1984, pp. 176-191.
11. Previously[6a], it has been proposed that the interactions between (1) and peptides in beer foam are stabilized by electrostatic (ionic) interactions between the anion of hop bitter acids such as (1) and protonated amino groups on the peptide.

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