Stepwise pH-Gradient Elution for the Preparative Separation of Natural Anthraquinones by Multiple Liquid-Liquid Partition

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Preparative-scale separation of substituted anthraquinones by multiple liquid-liquid partition was studied using isopropylmethyl ketone (IMK)/aqueous phosphate buffer (aq.) as the solvent system and the Hietala apparatus with 100 partition units as the partition equipment. The lower (aq.) phase was chosen as mobile, while the upper (IMK) phase remained stationary. Hence, the principle of stepwise pH-gradient elution could be utilized to separate the components in two complex mixtures of hydroxyanthraquinones and hydroxyanthraquinone carboxylic acids, isolated from the fungus *Dermocybe sanguinea*. In spite of the nonlinearity of the partition isotherms for these anthraquinones, implying deviations from the Nernst partition law, remarkable separations were achieved for the components in each mixture. Every anthraquinone carboxylic acid showed markedly irregular partition behavior, appearing in the effluent as two more or less resolved concentration zones. Such splitting was attributed to the formation of relatively stable sandwich-dimers, which were in a slow equilibrium with the monomers in the more nonpolar organic phase. At lower pH-values, only the polar monomers were distributed into the mobile aqueous phase and moved forward, whereas the nonpolar sandwich-dimers remained almost entirely in the stationary organic phase and lagged behind. When the pH of the mobile aqueous phase was raised high enough, the firmly linked dimers were monomerized and emerged from the apparatus as a second concentration profile. Intermolecular hydrogen bonding and π-π interaction between the π-systems of two anthraquinone molecules in a parallel orientation were considered responsible for the nonlinear and markedly irregular partition behavior of the natural anthraquinones studied. The nonlinearity of the partition behavior of the hydroxyanthraquinones lacking the carboxyl group, appeared merely as excessive broadening of the experimental concentration profile, which impaired the resolution between the components only insignificantly. Thus, *e.g.* the main components, dermocybin and emodin, could both be obtained from Separation 1 in a purity of at least 99%.