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ORIGINAL PAPER

Esterification equilibrium constants of arsonic and arsinic acids

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Abstract The esterification equilibrium constants between CD_3OD and five arsonic acids (methyl, pentyl, phenyl, 2,3-dipalmitoyloxypropyl, and 2,3,4-tripalmitoyloxybutyl) and one arsinic acid (cacodylic acid) were measured in the solvent system $CDCl_3/CD_3OD$ at 25.0 °C. Equilibrium concentrations of the diester, monoester, and free acid for each compound were calculated from the integrations of the corresponding ^1H NMR peaks. It was found that the nature of the organic part of the arsonic acids essentially did not affect the measured equilibrium constants which were around 0.114 and 0.014 for K_1 and K_2, respectively. However, the equilibrium constant K_1 of cacodylic acid was 0.053. Diesters and monoesters are very easily hydrolyzed, and in particular the diesters are extensively hydrolyzed even in the presence of tiny amounts of water. Thus, esters of arsonic or arsinic acids with monohydric alcohols are expected to be in very low concentrations inside cells, a conclusion that may be of interest to the "arsenic bacterium debate".

Keywords Bioinorganic chemistry · NMR spectroscopy · Main group compounds · Arsenic compounds · Equilibrium constants

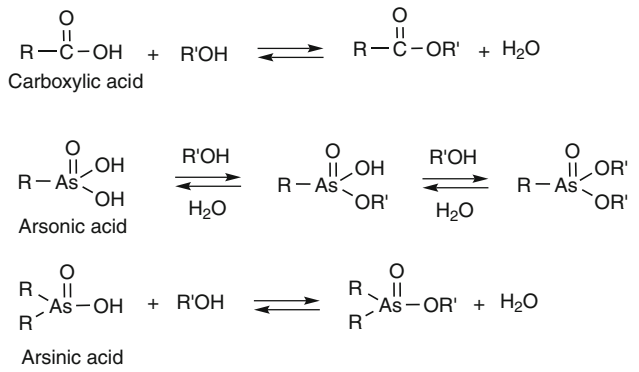
Introduction

Arsenic compounds are known for their toxicity [1] and they are also pollutants from certain industrial processes [2]. Without doubt, the ability of arsenic oxo-compounds to react with free thiols or enzymes bearing –SH groups is important for their biochemical reactions [3]. There are marine organisms capable of incorporating arsenic in water-soluble [4, 5] or lipid-soluble [5, 6] molecules having an As–C bond without apparent toxicity or, as recently claimed, to their DNA through an As–O–C bond [7]. Moreover, certain arsenic compounds, e.g., arsenic trioxide, show anticancer properties [8]. In order to better understand the role of arsenic compounds in living organisms, information on esterifications of arsenous, arsonic, and arsinic acids are necessary.

The reaction between an arsonic or arsinic acid with an alcohol leads to esterification in a way similar to the esterification of a carboxylic acid (Scheme 1).

However, the esterification of arsonic and arsinic acids proceeds much faster, because the bigger size of the arsenic atom compared to the carbon atom permits an easier entry of the alcohol [9]. The same applies for the hydrolysis of their esters [9].

Although a number of articles have been published by our group on the synthesis and properties of aliphatic



Scheme 1

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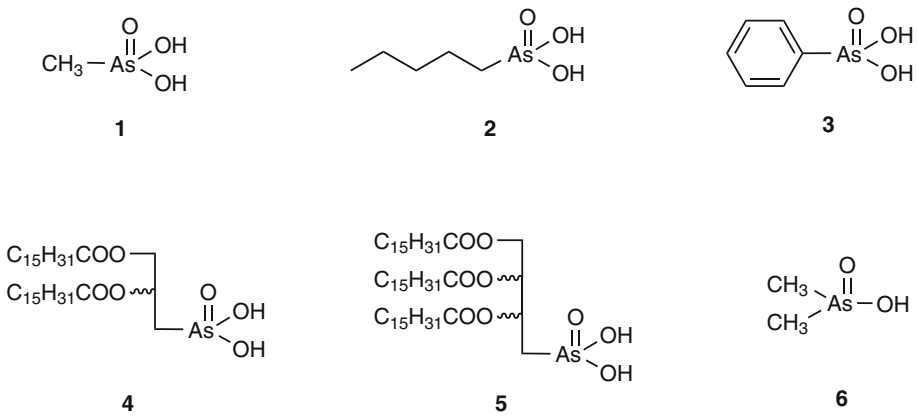


Fig. 1 Structures of the acids dealt with in this study

arsonic acids [10–13], no detailed studies were done on their esterification. The kinetics for the hydrolysis of arsenous triesters, $As(OR)_3$ [14], the equilibrium constants for the transesterification of arsenous triesters [3], and the rates of esterification of aromatic arsonic acids with certain alcohols [15] have been published but no quantitative data are available for the esterification equilibrium constants of arsonic or arsinic acids and this absence of relevant information has been noticed recently [16–18].

Various techniques are available for the estimation of esterification equilibrium constants, i.e., titrations [19], NMR measurements of various nuclei [20, 21], UV–Vis measurements [22], etc. These techniques are based on the direct or indirect determination of the concentration change of the reactants and/or products. Among them ^{1}H NMR is particularly suited because the change in concentrations of both reactants and products can usually be followed and the existence of by-products or stable intermediates can be revealed.

In this paper, the esterification equilibrium constants between deuterated methanol and five arsonic acids [methyl (**1**), pentyl (**2**), phenyl (**3**), 2,3-dipalmitoyloxypropyl (**4**), and 2,3,4-tripalmitoyloxybutyl (**5**)] and one arsinic acid [cacodylic acid (**6**)] are reported (Fig. 1). The organic parts on these acids were chosen in such a way as to permit the evaluation of the effect of parameters such as the alkyl group length, the aromatic versus aliphatic nature, and the arsonic versus arsinic group on the esterification equilibrium constants.

Results and discussion

It is known that arsenous acid, H_3AsO_3, reacts reversibly with alcohols. The reaction rates were found to be very fast with simple alcohols but became slower only with bulky ones like *tert*-butyl alcohol [3]. Similarly, arsonic and arsinic acids react readily with alcohols [15]. The structure of the produced esters depends on the mono- or dihydric

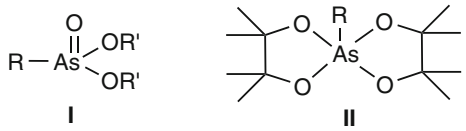


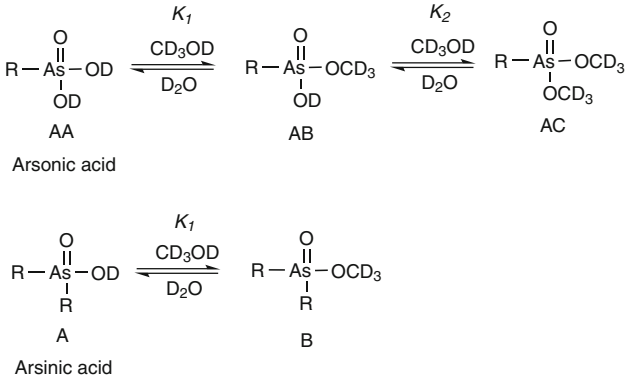
Fig. 2 General structures of produced esters

character of the alcohol. Monohydric alcohols give the expected diesters **I** [15], whereas in the case of 1,2-diols the diesters react further and give spiro-products of the general structure **II** [23] (Fig. 2).

To push the equilibrium towards the diester, either azeotropic distillation of water [24, 25] or large excess [15] of the monohydric alcohol under anhydrous conditions was used. Thus, the electrospray ionization mass spectrometry (ESI–MS) spectrum of ferrocenyl arsonic acid in methanol revealed only the presence of the monoester [26]. It is worth noting that under similar conditions the ESI–MS spectrum of ferrocenyl phosphonic acid showed no sign of esterification, because the free acid instead of the ester was observed. These results indicate that esterification of arsonic acids is very fast compared to the esterification of phosphonic acids. Interestingly, the spiropyran-type compounds **II** produced from 1,2-diols and arsonic acids are much less sensitive to hydrolysis [23]. The equations describing the reaction of deuterated arsonic and arsinic acids with CD_3OD are shown in Scheme 2.

Under the experimental conditions used in this work (mixtures of $CDCl_3/CD_3OD$ plus small quantities of added D_2O at 25.0 °C) it was found that both the esterification and the hydrolysis reactions were fast, similar to the results obtained in the case of arsenous acid [3]. Thus, addition of either CD_3OD (esterification) or D_2O (hydrolysis) to the solution of the arsonic acid in $CDCl_3/CD_3OD$ resulted in a fast equilibrium, as was revealed by the identical ^{1}H NMR spectra obtained after 2 min, 30 min, and 24 h.

It should be emphasized that accurate measurements of equilibrium constants are not an easy task. Many factors like pH, ionic strength, temperature, solvent system,



Scheme 2

parallel equilibria present, solubility limitations, etc. can affect the measured variables. In the present study, the temperature was constant at 25.0 °C and the initial concentration of the arsonic and arsinic acids was kept identical in all experiments permitting comparisons between the various acids despite the fact that not all of the above factors were precisely fixed.

Ionization of the arsonic and arsinic acids will result in the appearance of additional equations in Scheme 2. Arsonic acids are weak acids [27] and calculations for the concentration range used in our experiments gave approximately 20 % deprotonation in water. However, in the $CDCl_3/CD_3OD$ mixture (volume ratio 5:4) used in this work deprotonation should be less than 1 %, because it is known that dissociation is reduced by several orders of magnitude on going from water to organic solvents [28].

The solvent system can influence the equilibria in Scheme 2 not only because of ionization but also because of solubility changes. The choice of the solvent system of $CDCl_3/CD_3OD$ was based mainly on solubility considerations because all the studied acids, lipophilic or not, were soluble in this mixture.

The expressions for the esterification equilibrium constants shown in Scheme 2 can be written as

$$K_1 = \frac{[AB][D_2O]}{[AA][CD_3OD]} = \frac{n_{AB}}{n_{AA}}\frac{[D_2O]}{[CD_3OD]} = \frac{I_{AB}n_t}{I_{AA}n_t}\frac{[D_2O]}{[CD_3OD]} = \frac{I_{AB}[D_2O]}{I_{AA}[CD_3OD]} \quad (1)$$

$$K_2 = \frac{I_{AC}[D_2O]}{I_{AB}[CD_3OD]} \quad (2)$$

where I_{AA}, I_{AB}, and I_{AC} represent the ^{1}H NMR relative peak intensities for the peaks of R–CH_2–As(O)$(OD)_2$, R–CH_2–As(O)(OD)(OCD_3), and R–CH_2–As(O)$(OCD_3)_2$, respectively, and n_t represents the total amount, in moles, of the arseno compound added initially. Concentrations of free acid (AA), monoester (AB), and diester (AC) in the numerator and denominator have been replaced by amounts n_{AA}, n_{AB}, and n_{AC} in moles (because at each equilibrium point the total volume was the same).

In order to calculate the constants K_1 and K_2, at any equilibrium point, all the variables I_{AA}, I_{AB}, I_{AC}, $[D_2O]$, and $[CD_3OD]$ should be estimated. Although this is relatively easy for the variables I_{AA} and I_{AB} by integration of the respective peaks in the ^{1}H NMR spectra, it cannot be applied for the determination of $[D_2O]$ and $[CD_3OD]$.

Estimation of I_{AA}, I_{AB}, and I_{AC} under experimentally fixed $[D_2O]/[CD_3OD]$ concentration ratios has been used as a solution to this problem [21]. Thus, in a typical experiment known volumes of D_2O and CD_3OD were added into the NMR tube and, after equilibration, the I_{AA}, I_{AB}, and I_{AC} relative peak intensities were measured. The signals corresponding to the diester, monoester, and free acid were identified by their behavior during the stepwise addition of D_2O. Thus, the signal of the diester was reduced continuously, that of the monoester initially increased and then decreased (see Fig. 3), whereas the signal of the free acid continuously increased. In this case, the value of $[D_2O]/[CD_3OD]$ at each ratio can be easily calculated as follows:

$$\frac{[D_2O]}{[CD_3OD]} = \frac{n_{D_2O}}{n_{CD_3OD}} = \frac{V_{D_2O}d_{D_2O}/M_{D_2O}}{V_{CD_3OD}d_{CD_3OD}/M_{CD_3OD}} \quad (3)$$

where V_{CD_3OD} is the volume of CD_3OD added initially, V_{D_2O} corresponds to the total volumes of D_2O added each time, and d and M represent density and molecular weight, respectively. ^{1}H NMR spectra recorded for pentylarsonic acid (**2**) under these conditions are reproduced in Fig. 3.

^{1}H NMR signals for the acids **1**–**6** are shown in Fig. 4 and the related data are presented in Table 1.

Plots of $[D_2O]/[CD_3OD]$ versus I_{AA}/I_{AB} (or I_{AB}/I_{AC}) for different $[D_2O]/[CD_3OD]$ ratios gave linear relationships as shown in Fig. 5 for the case of methylarsonic acid (**1**).

The lines drawn by the linear least-squares method gave R^2 values close to unity and from the slopes of these lines the equilibrium constants K_1 or K_2 were calculated. Results for all the studied acids are summarized in Table 2.

It should be noted that in the case of 2,3,4-tripalmitoyloxybutylarsonic acid (**5**) integration of the ^{1}H NMR peaks is difficult (see Fig. 4) as a result of diastereoisomers present, and so the values obtained for K_1 and K_2 are subject to significant errors. Also, in the case of phenylarsonic acid (**3**) the order of chemical shifts of the signals AA, AB, and AC is reversed (see Fig. 4) and, as a result of the peak overlapping, additional experimental points were required to estimate the equilibrium constants. In particular, addition of D_2O to a total amount of at least 50 mm^3 was necessary to ensure that no diester was present and the ^{1}H NMR signals at around 7.50–7.65 were caused by the monoester and the free acid only.

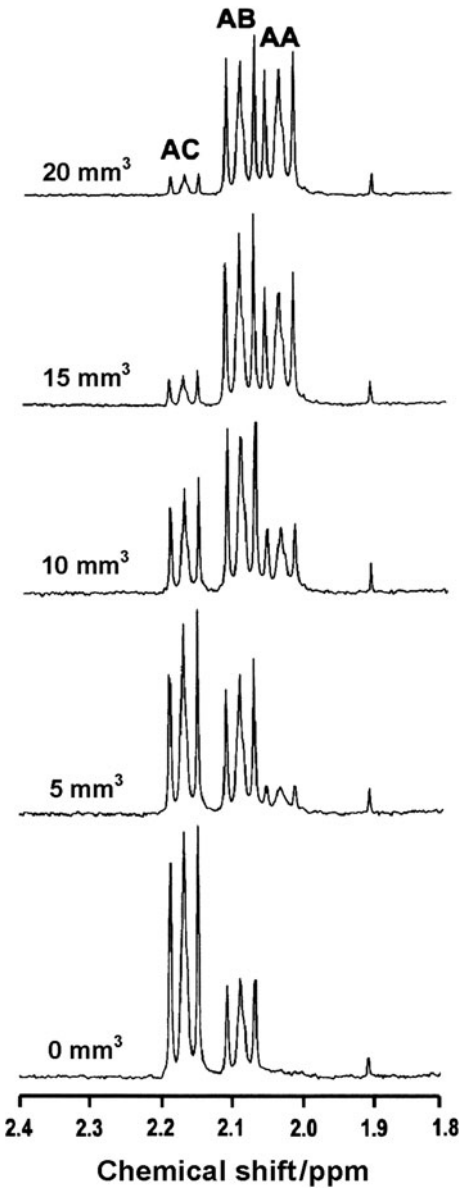


Fig. 3 Part of the ^{1}H NMR spectra of pentylarsonic acid (**2**, 2.5 nmol) in $CDCl_3/CD_3OD$ (500/400 mm^3) in the presence of added D_2O equal to 0, 5, 10, 15, and 20 mm^3. AA, AB, and AC correspond to C_4H_9–CH_2–As(O)$(OD)_2$, C_4H_9–CH_2–As(O)(OD)(OCD_3), and C_4H_9–CH_2–As(O)$(OCD_3)_2$ protons, respectively

In the experiments described above, there was concern for the very small amount of D_2O (5–40 mm^3) found to be necessary to shift the equilibrium towards the monoester and the free acid. Besides the relatively large experimental errors involved in the addition of such tiny amounts of D_2O, questions can be raised about the validity of the assumption that the $[CD_3OD]/[D_2O]$ ratio is fixed and, for example, it is not affected by the amount of water or methanol in the esterification/hydrolysis reactions. However, calculations showed that even 5 mm^3 of D_2O corresponds to 2.5×10^{-4} mol, whereas the total amount of arseno compound added was 2.5×10^{-6} mol, which is 100 times smaller. Therefore, the amount of water liberated by the esterification reaction should not be important. Indeed, as shown in Table 1, when the K_1 and K_2 values are recalculated taking into account this additional factor (presented as K_{1cor} and K_{2cor}, respectively) they are essentially identical to the values obtained before corrections. For the calculation of K_{1cor} and K_{2cor} the expression 1 was rewritten as follows:

$$\begin{aligned} K_1 &= \frac{I_{AB}[D_2O]}{I_{AA}[CD_3OD]} = \frac{I_{AB}}{I_{AA}}\frac{n_{D_2O}}{n_{CD_3OD}} \\ &= \frac{I_{AB}}{I_{AA}}\frac{(V_{D_2O}d_{D_2O}/M_{D_2O} + n_{AB} + 2n_{AC})}{(V_{CD_3OD}d_{CD_3OD}/M_{CD_3OD} - n_{AB} - 2n_{AC})} \\ &= \frac{I_{AB}}{I_{AA}}\frac{(V_{D_2O}d_{D_2O}/M_{D_2O} + I_{AB}n_t + 2I_{AC}n_t)}{(V_{CD_3OD}d_{CD_3OD}/M_{CD_3OD} - I_{AB}n_t - 2I_{AC}n_t)} \end{aligned} \quad (4)$$

where the additional factors correspond to water and methanol produced and consumed, respectively, during the two-step esterification.

The values of K_1 and K_2 (Table 2) show that the equilibria presented in Scheme 2 are shifted towards the monoesters (AB) and the free acids (AA) even in the presence of small amounts of water. Indeed, as is evident from Figs. 3 and 4, for the arsonic acids **1**–**5**, the presence of only 15 mm^3 of D_2O in 400 mm^3 of CD_3OD is enough to significantly diminish the concentration of the corresponding diesters (AC). Previous results [29], based on ^{1}H NMR data, showed that when longer alcohols like CD_3CD_2OD were used, sensitivity to water was further enhanced.

The esterification equilibrium constants are not significantly affected by the nature of the R group in arsonic acids. Therefore, aromatic and short or long aliphatic groups gave similar values for K_1 and K_2 of around 0.114 and 0.014, respectively. One exception seems to be the triester arsonic acid **5** but as mentioned above no safe conclusions can be drawn in this case because significant errors are present. When the arsonic acids are compared to the arsinic acid **6**, differences in K_1 were found. Thus, in the case of dimethylarsinic acid (**6**), the value obtained for K_1 was 0.053 which is about half of the values observed for the arsonic acids. A second arsinic acid (diphenyl arsinic acid) was synthesized and measured as well (results not shown) but correct integrations were not possible due to the extensive overlapping of the ^{1}H NMR signals in the aromatic region observed in this case.

Conclusions

We have reported herein that the esterification between arsonic and arsinic acids with CD_3OD and the hydrolysis of their esters with D_2O were both very fast reactions. ^{1}H NMR measurements revealed that the equilibria shown in Scheme 2 were shifted towards the monoesters and the free

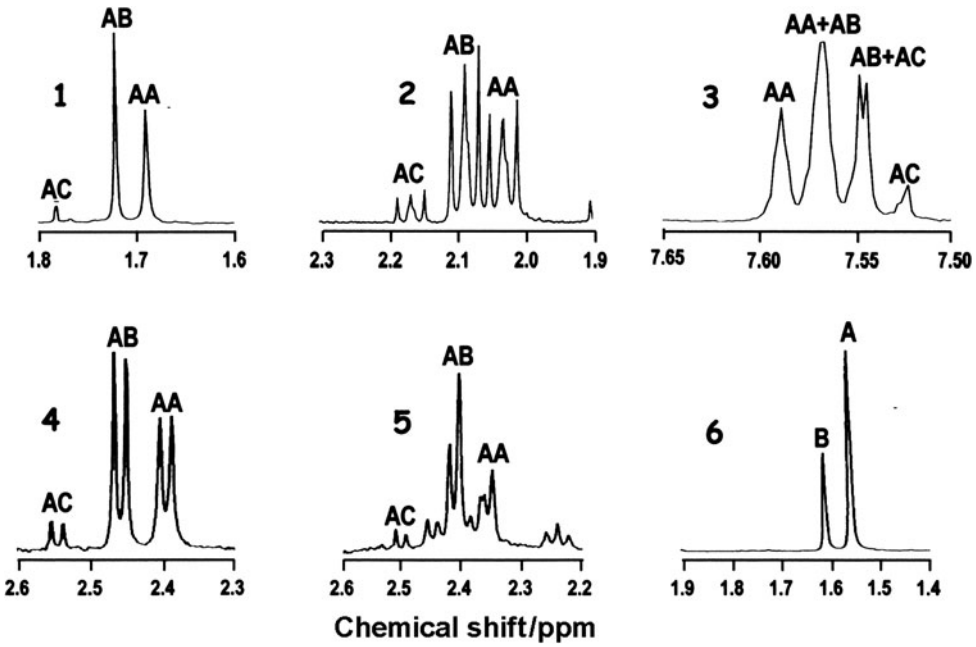


Fig. 4 ^{1}H NMR signals of the acids **1**–**6** (2.5 nmol), in $CDCl_3$/CD_3OD/D_2O (500/400/15 mm^3). AA = RCH_2As(O)$(OD)_2$, AB = RCH_2As(O)(OD)(OCD_3), and AC = RCH_2As(O)$(OCD_3)_2$. In the case of phenylarsonic acid (**3**) the signals correspond to the *ortho* protons of the phenyl group

Table 1 ^{1}H NMR chemical shifts and multiplicities in parenthesis of the acids **1**–**6** (2.5 nmol) in $CDCl_3$/CD_3OD/D_2O (500/400/15 mm^3)

Acid	R–CH_2–As(O) $(OD)_2$ AA	R–CH_2–As(O) (OD)(OCD_3) AB	R–CH_2–As(O) $(OCD_3)_2$ AC
1	1.69 (s)	1.72 (s)	1.78 (s)
2	2.04 (t)	2.09 (t)	2.17 (t)
3	7.58 (apparent d)	7.56 (apparent d)	7.53 (apparent d)
4	2.40 (apparent dd)	2.46 (apparent dd)	2.55 (apparent dd)
5	2.35 (apparent dd)	2.41 (apparent dd)	2.50 (apparent dd)
6	1.56 (s)	1.61 (s)	–

The term *apparent* indicates that the multiplicity of the signal is lower than the expected one because of accidental equivalence of some protons or values close to zero for some coupling constants. In the case of phenylarsonic acid (**3**) the signals refer to the *ortho* protons of the phenyl group

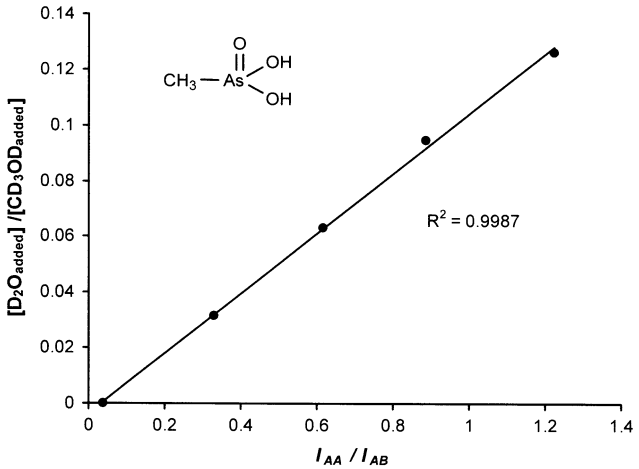


Fig. 5 Plot of $[D_2O_{added}]/[CD_3OD_{added}]$ versus I_{AA}/I_{AB} in the esterification of methylarsonic acid (**1**) with CD_3OD

acids even in the presence of very small amounts of water. Thus, the concentration of arsonic esters of the general type **I** inside cells should be negligible. This result may provide some additional insight into the recently raised "arsenic bacterium debate" [16–18]. The nature of the R group in the arsonic acids **1**–**5** does not seem to affect the esterification equilibrium constants. However, for cacodylic acid (**6**, an arsinic acid) the value of K_1 is about half of the values obtained for the arsonic acids **1**–**5**. Future experiments with other arsinic acids may disclose if this behavior is general or not.

Experimental

Dimethylarsinic acid (cacodylic acid) was from Serva. Methylarsonic acid was prepared from alkaline "Na_3AsO_3"

Table 2 Esterification equilibrium constants for arsonic and arsinic acids with CD_3OD calculated from various $[D_2O]/[CD_3OD]$ ratios

Acid	K_1	K^a_{1cor}	K_2	K^a_{2cor}
1	0.113 ± 0.002	0.113 ± 0.002	0.010 ± 0.001	0.010 ± 0.001
2	0.117 ± 0.006	0.116 ± 0.006	0.014 ± 0.002	0.013 ± 0.003
3	0.110 ± 0.006	0.111 ± 0.006	0.014 ± 0.002	0.014 ± 0.002
4	0.113 ± 0.004	0.113 ± 0.004	0.016 ± 0.001	0.015 ± 0.001
5	0.20[b]	0.20[b]	0.010[b]	0.010[b]
6	0.053 ± 0.001	0.053 ± 0.001	–	–

[a] Values were corrected for the amount of water liberated and the amount of CD_3OD consumed during the esterification reaction

[b] Significant errors are involved in this measurement because the ^1H NMR peaks are difficult to separate (see text)

and methyl iodide as $Me\text{-}AsO_3Na_2{\cdot}6H_2O$ [30] and converted to the free acid via the strong cation-exchange resin Dower AG50W-X8, eluting with water, evaporating (rotary 50 °C) the fractions with pH 2–3 and drying in vacuo. Phenylarsonic acid (97 %) was from Alfa Aesar. Pentylarsonic acid [13], 2,3-dipalmitoyloxypropylarsonic acid [10, 11], and 2,3,4-tripalmitoyloxybutylarsonic acid [12] were synthesized essentially according to literature methods. $CDCl_3$ (x_D = 99.8 %) with silver foil (w_{Ag} = 0.5 %) as stabilizer, CD_3OD (x_D = 99.8 %), and D_2O (x_D = 99.9 %) were from Aldrich.

^1H NMR spectra at 400 MHz were obtained on a Bruker Avance DPX spectrometer at 25.0 °C. All weighings were done on a 5-digit Mettler-Toledo balance, model XS205. $CDCl_3$ and CD_3OD were added with gas-tight Hamilton syringes of 500 mm^3, whereas D_2O was added with a gas-tight Hamilton syringe of 10 mm^3.

General experimental procedure

The arsenic compound (2.5 nmol) was dissolved in 500 mm^3 $CDCl_3$ in an NMR tube. CD_3OD (400 mm^3) was added, and the tube was tumbled until equilibrium was reached. In the cases of methylarsonic acid (**1**), 2,3-dipalmitoyloxypropylarsonic acid (**4**), 2,2,4-tripalmitoyloxybutylarsonic acid (**5**), and cacodylic acid (**6**), dissolution was complete only after the CD_3OD addition. ^1H NMR spectra showed that equilibrium was practically reached immediately after the CD_3OD addition. Then, increasing amounts of D_2O were added, in the order of total 5, 10, 15, 20, and 40 mm^3. After each addition the tube was tumbled and the ^1H NMR spectra were recorded.

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