Simple Methods for Determining Relative Stereochemistry of Kainoid Amino Acids by ^{1}H NMR Chemical Shifts

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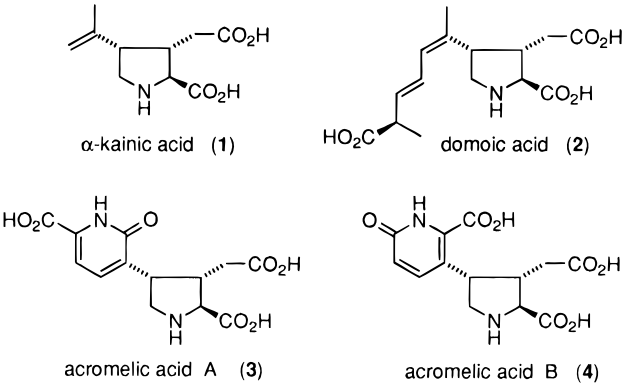
The kainoid amino acids are biologically important compounds because they show remarkable neuroexcitatory and excitotoxic activities. For exhibiting potent activity, the stereochemical relationship of the substituents on the pyrrolidine ring is crucial. We found simple methods for determining the relative stereochemistry of these compounds on the basis of the ^{1}H NMR chemical shifts of H-2 and H-4 in D_2O solution. The signals of H-2 appear at fields higher than 4.2 ppm when the compounds have 2,3-*trans* stereochemistry whereas, in the 2,3-*cis* compounds, they appear lower than 4.2 ppm, irrespective of the C-4 substituent. This criterion holds when the solution is in the range of pD 3−8. Moreover, when an epimeric pair at C-2 is available and the spectra are recorded at the same or nearly equal pD, the H-2 chemical shift of the 2,3-*trans* isomer is higher than that of the corresponding 2,3-*cis* isomer. Similarly, the relative stereochemistry between C-3 and C-4 can be determined from the chemical shift of H-4. The signals of H-4 of the 3,4-*cis* isomers appear at lower fields than those of the corresponding 3,4-*trans* isomers in each pair of C-4 epimers when the spectra are recorded at the same or nearly equal pD. This holds for the compounds bearing an unsaturated substituent at C-4. All these phenomena can be rationalized by the anisotropic effect of the π-electron system in the C-2 and C-4 substituents.

Introduction

The kainoid amino acids are a class of compounds related to α-kainic acid (**1**) both in chemical structure and in biological activities and members of excitatory amino acids, which includes domoic acid (**2**) and acromelic acids A (**3**) and B (**4**). α-Kainic acid was originally isolated as an anthelmintic principle of the seaweed *Digenea simplex*[1] and later found to show potent neuroexcitatory and excitotoxic activity for the central nervous systems in vertebrates.[2] Domoic acid is also an anthelmintic component of the red algae *Chondria armata*[3] and a highly potent neurotoxin as well.[4] It was identified as the toxin responsible for an outbreak of the amnesic shellfish poisoning in eastern Canada in 1987 and the deaths of seabirds in California in 1991 and demonstrated to cause neurologic degeneration such as memory impairment to man.[5] These incidents have prompted a renewed interest in the chemistry[6] and pharmacology[7] of domoic acid. Acromelic acids, which we have recently isolated,[8] are the toxic principles of the poisonous mushroom *Clitocybe acromelalga* and proved to be the most potent agonist of excitatory amino acids in both vertebrates and invertebrates.[9]



The excitatory amino acids have attracted much attention because their actions are mediated by the glutamate receptors. Glutamic acid is a major excitatory

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neurotransmitter in mammalian central nervous systems, and the glutamate receptors are implicated in higher neural functions such as memory and learning, and in some neurological disorders such as epilepsy and Huntingtons' disease.[10] Therefore, extensive studies have focused on the glutamate receptors in recent years.[11] In particular, molecular cloning studies have made great advances in understanding the structures and properties of the glutamate receptors.[12] At present, the glutamate receptors are categorized into two major groups, the ionotropic receptor and the metabotropic receptor, on the basis of their pharmacological and biochemical properties, and the former is further divided into three subtypes according to the sensitivity to the agonists: NMDA (*N*-methyl-D-aspartate), AMPA ((*S*)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate), and kainate receptors.[13] Of these, the chemistry and pharmacology of the NMDA receptor is the most documented, but little is known about those of the kainate receptor. The kainoid amino acids, which should interact with the kainate receptor, would be useful for investigating the chemistry, pharmacology, and function of the kainate receptor.

It is important to determine the stereochemistry of the kainoids both from a chemical and pharmacological point of view since the studies on the structure−activity relationships of the kainoids have shown that the stereochemical relationship of the substituents on the pyrrolidine ring is crucial for exhibiting potent activity; the 2,3-*trans*-3,4-*cis* compounds show potent activity, whereas it is dramatically reduced in the 3-epi (2,3-*cis*) or 4-epi (3,4-*trans*) isomers.[14] In the structural elucidations of acromelic acids, we deduced the relative configurations by comparison of their ^{1}H NMR spectra with those of other kainoids, which were finally corroborated by their syntheses.[8] During these studies, we suggested an empirical rule for determining relative stereochemistry based on the ^{1}H NMR spectra. Thus the relative configuration between C-2 and C-3 can be simply determined from the chemical shift of H-2 in D_2O solution; the signals of H-2 appear at fields higher than 4.2 ppm when the compounds have 2,3-*trans* stereochemistry whereas, in the 2,3-*cis* compounds, they appear lower than 4.2 ppm, irrespective of the substituent at C-4. On the other hand, during the synthetic studies on kainoid analogs, Goldberg and co-workers pointed out that the H-4 signals of the 3,4-*cis* isomers appear at lower fields than those of the corresponding 3,4-*trans* isomers in each pair of C-4 epimers.[15] This could be an empirical rule for determining relative stereochemistry between C-3 and C-4 of the kainoids, but the available data were only for two cases. Later we have noted that this would have wide applicability and the spectra must be measured at the same pD.[16]

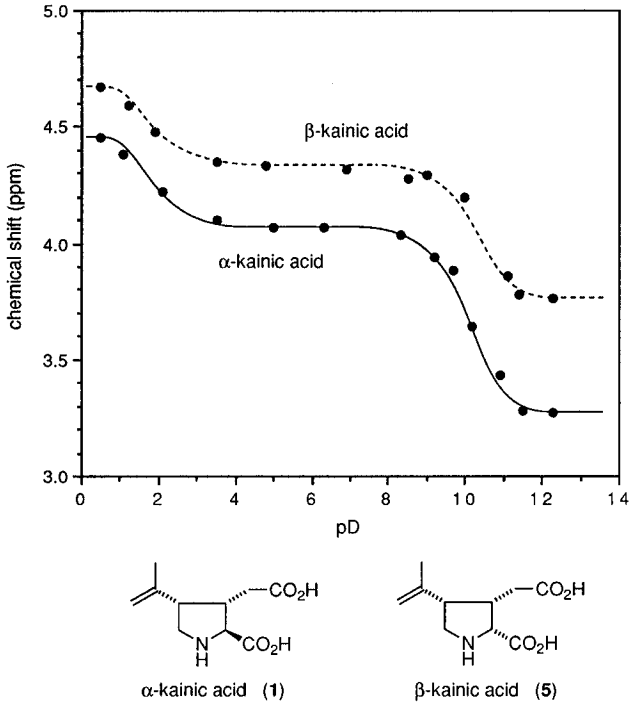

Figure 1. H-2 chemical shifts at various pD.

If the above empirical rules were generally valid, they would become simple methods for determining the stereochemistry of the kainoids. The recent efforts toward isolation of natural congeners[17] and syntheses of analogs,[18] which have yielded more than 50 novel kainoids so far, now make it possible to explore the validity of the rules in detail. We describe here the scope and limitation of the rules and discuss the origin of these phenomena.

Results and Discussion

Relative Stereochemistry between C-2 and C-3. For recording the ^{1}H NMR spectra of amino acids in D_2O,

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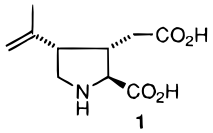
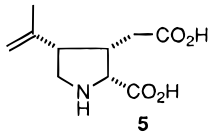
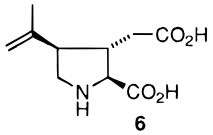
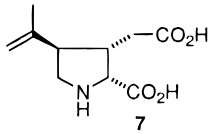
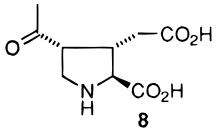
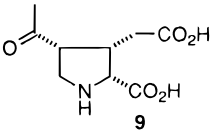
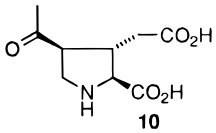
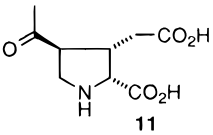
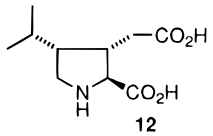
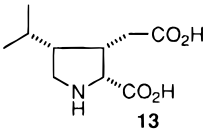
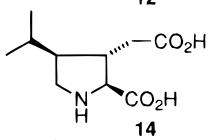
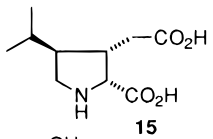
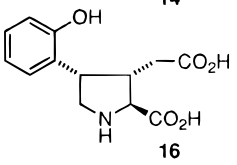
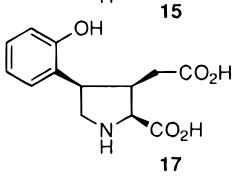
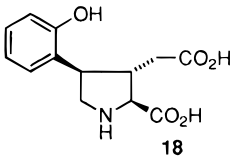
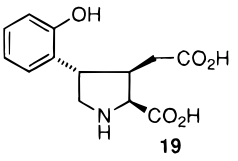
Table 1. H-2 Chemical Shifts of the C-2 Epimeric Pairs

Entry	2,3-*trans* isomers	δ (ppm) (pD)	2,3-*cis* isomers	δ (ppm) (pD)	Δδ [a]
1	 **1**	4.10 (3.5)	 **5**	4.36 (3.5)	+0.26
2	 **6**	3.92 (3.6)	 **7**	4.32 (3.3)	+0.40
3	 **8**	3.99 (3.0)	 **9**	4.36 (3.1)	+0.37
4	 **10**	3.95 (3.1)	 **11**	4.22 (3.0)	+0.27
5	 **12**	4.14 (3.1)	 **13**	4.23 (3.4)	+0.09
6	 **14**	4.00 (3.4)	 **15**	4.27 (3.4)	+0.27
7	 **16**	4.16 (4~5)	 **17**	4.52 (4.9)	+0.36
8	 **18**	4.07 (5.2)	 **19**	4.50 (4.3)	+0.43

[a] $\Delta\delta = \delta_{2,3\text{-}cis} - \delta_{2,3\text{-}trans}$

care must be taken about solution pD because the chemical shift of each proton varies as a function of pD due to the ionization states of the amino and carboxyl groups.[19] We, therefore, examined the pD dependency of the H-2 chemical shifts for the kainoid amino acids as represented by α-kainic acid (**1**) and *β*-kainic acid (**5**)[20] for the 2,3-*trans* isomer and 2,3-*cis* isomer, respectively. The results are summarized in Figure 1. As previously reported,[19a] the H-2 chemical shifts are fairly constant over the range of pD 3−8; they appeared within *δ* 4.0−4.1 and 4.3−4.4 for **1** and **5**, respectively. Accordingly, these data indicated that the "*δ* 4.2 criterion" for determining the relative stereochemistry between C-2 and C-3 should hold when the solution is in the pD range 3−8.

In order to corroborate the above results, we next examined the H-2 chemical shift of a variety of kainoids in the appropriate pD range. These include the isomers of kainic acid (**6** and **7**), kainic acid norketone (**8**−**11**), and dihydrokainic acid (**12**−**15**). All these compounds have been prepared during the structural elucidations of kainic acid.[21] Table 1 shows the results (entries 1−6) along with the reported data of the phenol analogs **16**−**19**[14d] (entries 7 and 8). In all the 2,3-*trans* compounds, the signals of H-2 appeared at higher than 4.2 ppm whereas, in all the 2,3-*cis* compounds, they appeared at lower than 4.2 ppm, irrespective of the C-4 substituent, which was in accord with the "*δ* 4.2 criterion". Consequently, the empirical rule we previously suggested for

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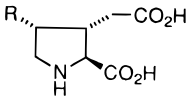
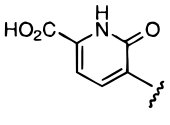
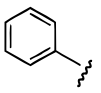
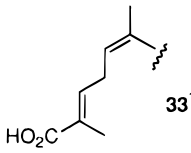
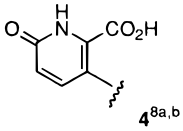
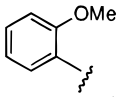
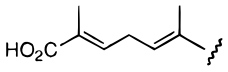
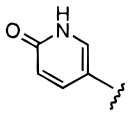
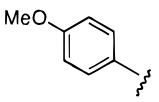
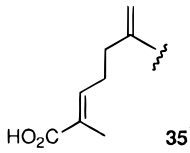
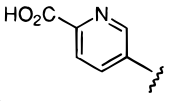
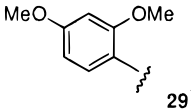
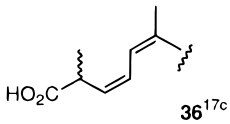
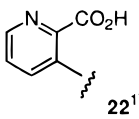
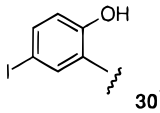
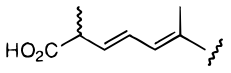
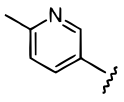
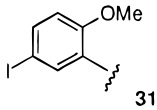
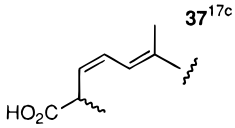
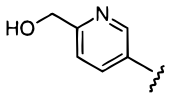
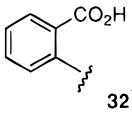
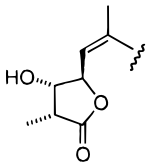
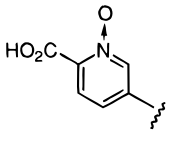
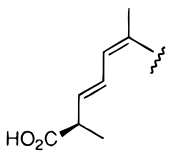
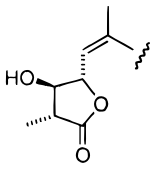
(21) (a) Murakami, S.; Takemoto, T.; Tei, Z.; Daigo, K. *J. Pharm. Soc. Jpn.* **1955**, *75*, 866. (b) Morimoto, H. *Ibid.* **1955**, *75*, 909. (c) Murakami, S.; Takemoto, T.; Tei, Z.; Daigo, K.; Takagi, N. *Ibid.* **1955**, *75*, 1252. (d) Morimoto, H.; Nakamori, R. *Ibid.* **1956**, *76*, 26. (e) Nakamori, R. *Ibid.* **1956**, *76*, 275.

Table 2. H-2 Chemical Shifts of the 2,3-*trans*-3,4-*cis*-Kainoids



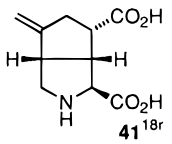
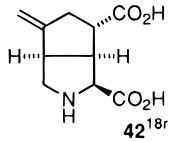
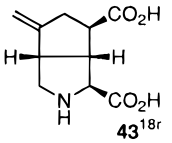
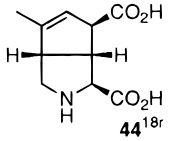
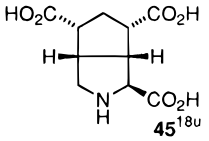
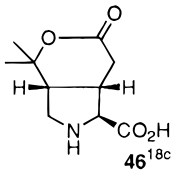
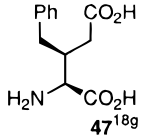
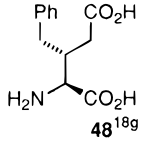
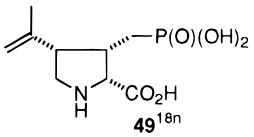
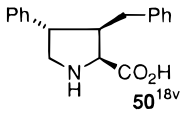
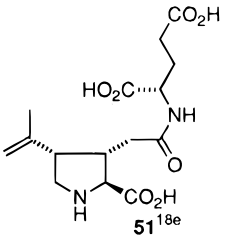
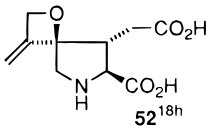
R	δ (ppm) (pD)	R	δ (ppm) (pD)	R	δ (ppm) (pD)
 **3**[8a,b]	4.13 (-)	 **26**[16]	4.13 (3.8)	 **33**[17a]	3.96 (-)
 **4**[8a,b]	4.14 (-)	 **27**[16]	4.13 (4.0)	 **34**[17a]	4.04 (-)
 **20**[17d]	3.78 (-)	 **28**[16]	4.08 (4.5)	 **35**[17a]	4.08 (-)
 **21**[17e, 18m]	4.14 (-)	 **29**[16]	4.10 (4.2)	 **36**[17c]	4.15 (1.9)
 **22**[17e]	4.23 (-)	 **30**[18t]	4.02 (-)	 **37**[17c]	4.36 (1.8)
 **23**[18m]	3.98 (-)	 **31**[18t]	3.93 (-)	 **38**[17c]	4.39 (1.6)
 **24**[18m]	4.08 (-)	 **32**[18t]	4.03 (-)	 **39**[17b]	3.97 (-)
 **25**[18m]	4.08 (-)	 **2**[17c]	3.98 (3.4)	 **40**[17b]	4.03 (-)

determining the relative configuration of C-2 and C-3 turned out to be generally valid and holds when the pD of the solution is in the pD range of 3−8, irrespective of the C-4 substituent.

It is notable for all cases in Table 1 that, regardless of the C-4 substituent, the H-2 chemical shifts of the 2,3-*trans* compounds are higher than those of the corresponding C-2 epimers. In other words, the $\Delta\delta$ (defined as $\delta_{2,3\text{-}cis} - \delta_{2,3\text{-}trans}$) gives positive values in all cases. Additionally, the pD dependency experiments showed that the H-2 chemical shifts of the C-2 epimeric isomers varied in almost a parallel fashion with each other over the whole pD range (Figure 1). Taking these facts into consideration, a new empirical rule can be suggested, that is, when a pair of C-2 epimers are available and the spectra are recorded at the same or nearly equal pD, the H-2 chemical shift of the 2,3-*trans* isomer is higher than that of the corresponding 2,3-*cis* isomer, irrespective of the C-4 substituent.

The origin of the above criteria can arise from the anisotropic effect of the α-carboxyl group. In the 2,3-*cis* compounds, the π-plane of the α-carboxyl group would be perpendicular to the pyrrolidine ring due to restricted rotation caused by the *cis*-oriented C-3 substituent. As a result, H-2 should lie on the π-plane of the α-carboxyl group, being deshielded by the π-electron system, and hence, the signal of H-2 should shift downfield. In contrast, in the 2,3-*trans* compounds, there would be no

Table 3. H-2 Chemical Shifts of the Miscellaneous Kainoid Analogs

structure	δ (ppm) (pD)	structure	δ (ppm) (pD)
 **41**[18r]	3.94 (3.7)	 **42**[18r]	4.25 (4.0)
 **43**[18r]	4.00 (3.2)	 **44**[18r]	3.94 (3.3)
 **45**[18u]	4.27 (2N DCl)	 **46**[18c]	3.98 (-)
 **47**[18g]	3.69 (-)	 **48**[18g]	3.84 (-)
 **49**[18n]	4.48 (-)	 **50**[18v]	4.41 (-)
 **51**[18e]	4.15 (-)	 **52**[18h]	3.96 (-)

or very little, if any, such deshielding effect on H-2 because the α-carboxyl group can freely rotate or the π-plane of the α-carboxyl group can be parallel to the pyrrolidine ring due to hydrogen bonding with the amino group.

Thus the two simple methods, the δ 4.2 criterion method and the $\Delta\delta$ method, are devised for determining the relative stereochemistry between C-2 and C-3 of the kainoids on the basis of the ^{1}H NMR chemical shifts. These two methods would be complementary to each other; when only a single isomer is available, usually for natural products, the δ 4.2 criterion method would be useful, whereas the newly devised rule, the $\Delta\delta$ method, would be applicable for synthetic analogs which are often obtained as a mixture of stereoisomers. The ^{1}H NMR data of a number of kainoids have been reported in the literature, so that utility of the above-devised methods can be checked. Table 2 summarizes the structures and the reported values of the H-2 chemical shifts of the currently available natural congeners and synthetic analogs of acromelic acid and domoic acid. Since all these compounds have 2,3-*trans* stereochemistry and only a single isomer is available, the δ 4.2 criterion method is applicable. For the cases in which the pD is specified, the data satisfy the criterion, but even for the cases in which the pD is unspecified, they appear to be in good agreement with the criterion. This is probably due to the fact that a D_2O solution of kainoids usually shows the pD value close to the isoelectric point (pD 3−5), so that it is in the appropriate pD range. Indeed, the D_2O solutions of both **1** and **5** (5 mg in 0.5 mL of D_2O) were at pD 3.5. In any case, however, the pD of the solution must be properly adjusted; otherwise the conclusion is ambiguous. Table 3 summarizes the data of other kainoid analogs. Of these, only the bicyclic analogs **41**−**46** follow the criterion. The acyclic or seco analogs **47** and **48** should not follow the criterion because they do not have a pyrrolidine ring. The validity of the analogs

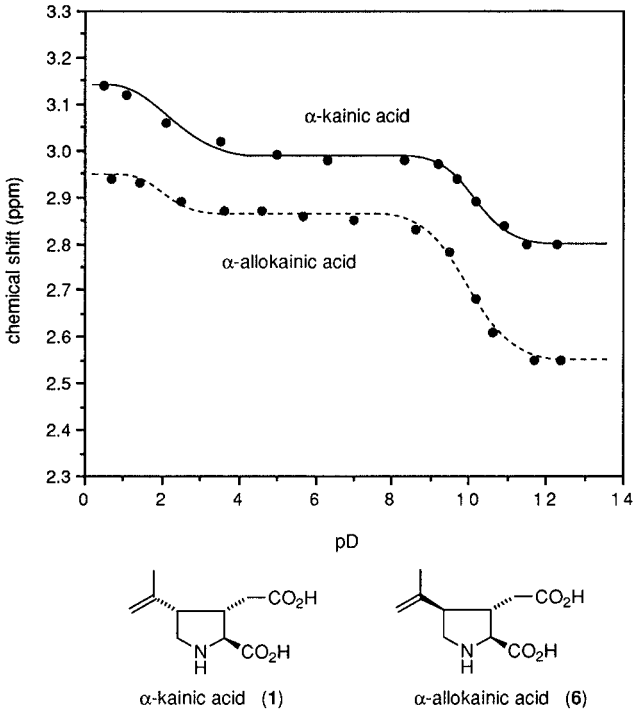


Figure 2. H-4 chemical shifts at various pD.

having a modified C-3 side chain (**49**−**51**) and the C-4-disubstituted analog **52** is ambiguous since the pD is not specified.

Relative Stereochemistry between C-3 and C-4. Similar to the above section, we first examined the pD dependency of the H-4 chemical shifts for α-kainic acid (**1**) and α-allokainic acid (**6**) as representative for the 3,4-*cis* and 3,4-*trans* isomers, respectively. As shown in Figure 2, the H-4 chemical shifts of **1** were lower than those of **6** at the corresponding pD over the whole range. Accordingly, the empirical rule suggested by Goldberg and co-workers would hold for the whole pD range, if the spectra are recorded at the same or nearly equal pD.

In order to corroborate the above results, the C-4 chemical shifts of a variety of kainoids (**5**−**19**) were next examined. Table 4 shows the results along with the $\Delta\delta$ values (defined as $\delta_{3,4\text{-}cis} - \delta_{3,4\text{-}trans}$). The majority of the $\Delta\delta$ values were positive, but those of the dihydrokainic acid isomers (entries 5 and 6) were negative, suggesting that this rule holds only when the compounds have an unsaturated substituent at C-4. Consequently, the rule is defined as follows: When a pair of C-4 epimers having an unsaturated substituent at C-4 is available and the spectra are recorded at the same or nearly equal pD, the H-4 chemical shift of the 3,4-*trans* isomer is higher than that of the corresponding 3,4-*cis* isomer.

This is basically the same as the $\Delta\delta$ method for the 2,3-stereochemistry as described in the previous section. Accordingly, this phenomena can be likewise rationalized by the anisotropic effect of the π-electron system in the C-4 substituent. Thus, in the 3,4-*cis* compounds, H-4 should lie on the π-plane of the C-4 substituent, resulting in a downfield shift of the H-4 signal, but there would be no such effect on H-4 for the 3,4-*trans* isomers.

This method would be especially useful for synthetic analogs since they are frequently obtained as a mixture of stereoisomers. Indeed, Baldwin and co-workers applied this method to determine the 3,4-stereochemistry of the 4-aryl acromelic acid analogs which were obtained as a mixture of C-4 epimers.[18q] Any criterion similar to the δ 4.2 criterion for the 2,3-stereochemistry would not be devised because the anisotropic effect degree varies and depends on the structure of the C-4 substituent as shown in Table 4.

Conclusions

In this study, we have established the validity, scope, and limitation of the empirical rules which have been previously suggested for determining the relative stereochemistry of the kainoids. As described here, these methods are quite simple to use and easy to judge by taking ^{1}H NMR spectra in D_2O (TSP as an internal standard) with specified pD and merely reading the chemical shifts of H-2 and H-4. The NOE experiment is an ordinary method for determining stereochemistry in general, but it is not always decisive for the kainoids; for example, no NOE is observed between H-3 and H-4 even when they are *cis*-oriented to each other.[22] In addition, it has been known that the coupling constant of the protons on a 5-membered ring is of no use for determining relative stereochemistry due to the flexibility of the ring conformation. Consequently, the methods described here should be the most reliable, except for the X-ray crystallographic analysis, for determining the relative stereochemistry of the kainoid amino acids.

Experimental Section

General. Melting points are uncorrected. ^{1}H NMR spectra were recorded in D_2O solution with TSP [3-(trimethylsilyl)-propionic−*2,2,3,3-d*$_4$ acid, sodium salt] as the internal standard. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and hertz, respectively. Acidity was measured directly in the NMR tubes using a glass microelectrode. The pD values correspond to the reading on a pH meter calibrated with pH 4 and 7 standard buffers in H_2O and are not corrected for the deuterium isotope effect.[23] The pD of the sample solution was adjusted by the addition of 2N DCl or 2 N NaOD. All the compounds **5**−**15** are known in literature[20,21] and prepared from α-kainic acid according to the reported procedures with slight modifications.

α-Kainic acid (1): ^{1}H NMR (400 MHz, pD 3.5) δ 1.76 (3H, s), 2.38 (1H, dd, J = 8.1, 16.5), 2.47 (1H, dd, J = 6.2, 16.5), 3.02 (1H, dt, J = 11.7, 7.3), 3.08 (1H, dddd, J = 3.3, 6.2, 7.3, 8.1), 3.43 (1H, t, J = 11.7), 4.10 (1H, d, J = 3.3), 4.76 (1H, s), 5.05 (1H, s); $\delta_{H\text{-}2}$ (pD) 4.45 (0.5), 4.38 (1.1), 4.22 (2.1), 4.10 (3.5), 4.07 (5.0), 4.07 (6.3), 4.04 (8.3), 3.94 (9.2), 3.88 (9.7), 3.64 (10.2) 3.43 (10.9), 3.28 (11.5), 3.27 (12.3); $\delta_{H\text{-}4}$ (pD) 3.14 (0.5), 3.12 (1.1), 3.06 (2.1), 3.02 (3.5), 2.99 (5.0), 2.98 (6.3), 2.98 (8.3), 2.97 (9.2), 2.94 (9.7), 2.89 (10.2), 2.84 (10.9), 2.80 (11.5), 2.80 (12.3).

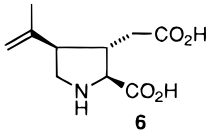
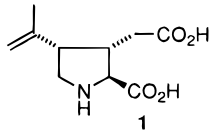
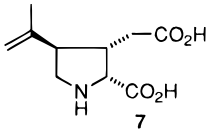
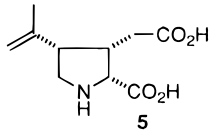
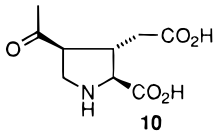
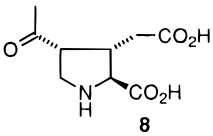
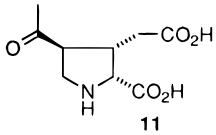
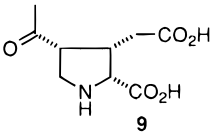
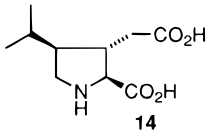
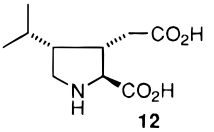
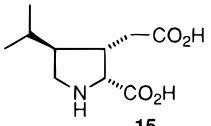
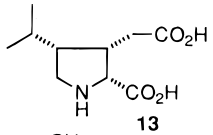
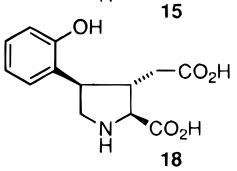
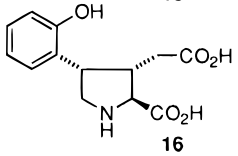
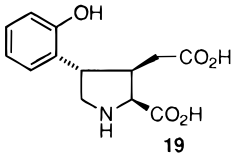
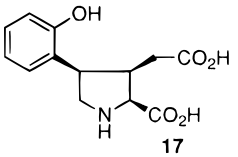
***β*-Kainic acid (5):** mp 241−243 °C dec; $[\alpha]_D$ +44.0° (*c* 0.28, H_2O) [lit.[21b] mp 244 °C dec; $[\alpha]_D$ +45.0° (*c*, 1.0, H_2O)]; IR (Nujol) 3540, 3140, 2920, 1695, 1620, 1570, 1425, 1380, 1335, 1275, 1235, 1205, 1185, 910, 790, 750 cm^{-1}; ^{1}H NMR (400 MHz, pD 3.5) δ 1.78 (3H, s), 2.23 (1H, dd, J = 6.2, 16.9), 2.34 (1H, dd, J = 7.0, 16.9), 3.16 (1H, ddd, J = 6.23, 8.4, 12.1), 3.30 (1H, dq, J = 7.0, 6.2), 3.45 (1H, t, J = 12.1), 3.51 (1H, dd, J = 8.4, 12.1), 4.35 (1H, d, J = 6.2), 4.80 (1H, s), 5.06 (1H, s); $\delta_{H\text{-}2}$ (pD) 4.67 (0.5), 4.59 (1.2), 4.48 (1.9), 4.35 (3.5), 4.33 (4.8), 4.32 (6.9), 4.28 (8.5), 4.29 (9.0), 4.20 (10.0), 3.86 (11.1), 3.78 (11.4), 3.76 (12.3); HRMS (FAB) *m/z* 214.1075 (M + H)$^+$ calcd for $C_{10}H_{16}O_4N$, 214.1079.

α-Allokainic acid (6): mp 235−237 °C dec; $[\alpha]_D$ + 11.0° (*c* 0.10, H_2O) [lit.[21c] mp 237−238 °C dec; $[\alpha]_D$ +6.7° (*c* 3.006, H_2O)]; IR (Nujol) 3580, 3160, 2940, 1730, 1700, 1630, 1580,

(22) Hashimoto, K. Ph.D. Thesis, Hokkaido University, 1993.

(23) (a) Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188. (b) Thibaudeau, C.; Plavec, J.; Chattopadhyaya, J. *J. Org. Chem.* **1996**, *61*, 266. (c) Jaroszewski, J. W.; Matzen, L.; Frølund, B.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1996**, *39*, 515.

Table 4. H-4 Chemical Shifts of the C-4 Epimeric Pairs

Entry	3,4-*trans* isomers	δ (ppm) (pD)	3,4-*cis* isomers	δ (ppm) (pD)	Δδ [a]
1	 **6**	2.87 (3.6)	 **1**	3.02 (3.5)	+0.15
2	 **7**	2.74 (3.3)	 **5**	3.16 (3.5)	+0.40
3	 **10**	3.39 (3.1)	 **8**	3.81 (3.0)	+0.42
4	 **11**	3.49 (3.0)	 **9**	3.87 (3.1)	+0.38
5	 **14**	2.09 (3.4)	 **12**	1.98 (3.1)	-0.11
6	 **15**	2.27 (3.4)	 **13**	2.21 (3.4)	-0.06
7	 **18**	3.70 (5.2)	 **16**	3.93 (4~5)	+0.23
8	 **19**	3.53 (4.3)	 **17**	4.13 (4.9)	+0.50

[a] $\Delta\delta = \delta_{3,4\text{-}cis} - \delta_{3,4\text{-}trans}$

1460, 1400, 1325, 1280, 1200, 1170, 1100, 1055, 925, 845 cm^{-1}; ^1H NMR (400 MHz, pD 3.6) δ 1.72 (3H, s), 2.62 (1H, m), 2.64 (1H, m), 2.78 (1H, m), 2.87 (1H, dt, J = 8.1, 11.7), 3.31 (1H, t, J = 11.7), 3.52 (1H, dd, J = 8.1, 11.7), 3.92 (1H, d, J = 9.2), 4.98 (2H, s); $\delta_{H\text{-}4}$ (pD) 2.94 (0.7), 2.93 (1.4), 2.89 (2.5), 2.87 (3.6), 2.87 (4.6), 2.86 (5.7), 2.85 (7.0), 2.83 (8.6), 2.78 (9.5), 2.68 (10.2), 2.61 (10.6), 2.55 (11.7), 2.55 (12.4); HRMS (FAB) *m/z* 214.1075 $(M + H)^+$ calcd for $C_{10}H_{16}O_4N$, 214.1079.

***β*-Allokainic acid (7):** mp 226−228 °C dec; $[\alpha]_D$ −21.7° (*c* 0.18 H_2O) [lit.[21c] mp 240 °C dec; $[\alpha]_D$ −21.1° (*c* 1.420, H_2O)]; IR (Nujol) 3300, 3200, 2920, 1735, 1610, 1570, 1455, 1420, 1330, 1260, 1210, 1195, 1170, 1140, 935, 910, 860, 775 cm^{-1}; ^1H NMR (400 MHz, pD 3.3) δ 1.73 (3H, s), 2.44 (1H, dd, J = 9.0, 17.0), 2.53 (1H, dd, J = 6.0, 17.0), 2.74 (1H, dt, J = 8.5, 10.5), 2.87 (1H ddt, J = 6.0, 10.5, 9.0), 3.25 (1H, dd, J = 10.5, 12.0), 3.65 (1H, dd, J = 8.5, 12.0), 4.32 (1H, d, J = 9.0), 4.97 (1H, s), 5.02 (1H, s); HRMS (FAB) *m/z* 214.1066 $(M + H)^+$ calcd for $C_{10}H_{16}O_4N$, 214.1079.

α-Kainic acid norketone (8): mp 187−188 °C dec; $[\alpha]_D$ +69.3° (*c* 0.27, H_2O) [lit.[21e] mp 184 °C dec; $[\alpha]_D$ +69.0° (*c* 1.0, H_2O)]; IR (Nujol) 3080, 2940, 2860, 1720, 1705, 1645, 1470, 1415, 1380, 1310, 1255, 1200 cm^{-1}; ^1H NMR (400 MHz, pD 3.0) δ 2.30, (3H, s), 2.49 (1H, dd, J = 9.3, 17.1), 2.73 (1H, dd, J = 5.9, 17.1), 3.14 (1H, ddt, J = 5.9, 9.3, 6.8), 3.57 (1H, dd, J = 6.5, 11.5), 3.55 (1H, dd, J = 6.8, 11.9), 3.81 (1H, dt, J = 5.9, 6.8), 3.99 (1H, d, J = 6.8); HRMS (FAB) *m/z* 216.0867 $(M + H)^+$ calcd for $C_9H_{14}O_5N$, 216.0872.

***β*-Kainic acid norketone (9):** mp 191−192 °C dec; $[\alpha]_D$ +32.3° (*c* 0.22, H_2O) [lit.[21e] mp 192 °C dec; α_D +62.0° (*c* 1.0, H_2O)]; IR (Nujol) 2900, 1715, 1615, 1460, 1380, 1170, 725 cm^{-1}; ^1H NMR (400 MHz, pD 3.1) 2.31 (3H, s), 2.41 (2H, d, J = 6.8), 3.46 (1H, dq, J = 6.3, 6.8), 3.48 (1H, dd, J = 8.3, 12.2), 3.67 (1H, dd, J = 9.4, 12.2), 3.87 (1H, ddd, J = 6.8, 8.3, 9.4), 4.36 (1H, d, J = 6.3); HRMS (FAB) *m/z* 216.0879 $(M + H)^+$ calcd for $C_9H_{14}O_5N$, 216.0872.

α-Allokainic acid norketone (10): mp 203−205 °C dec; $[\alpha]_D$ −18.8° (*c* 0.24, H_2O) [lit.[21e] mp 205 °C dec; $[\alpha]_D$ −17.5° (*c* 1.0, H_2O)]; IR (Nujol) 3200, 2930, 2860, 1740, 1715, 1615, 1460, 1420, 1395, 1360, 1315, 1240, 1225, 1195, 1170, 1085, 880 cm^{-1}; ^1H NMR (400 MHz, pD 3.1) δ 2.30 (3H, s), 2.63 (1H, dd, J = 9.3, 16.1), 2.81 (1H, dd, J = 4.9, 16.1), 3.04 (1H, ddt, J = 4.9, 9.3, 5.9), 3.39 (1H, dt, J = 7.8, 5.9), 3.60 (1H, dd, J = 7.8, 12.2), 3.68 (1H, dd, J = 5.9, 12.2), 3.95 (1H, d, J = 5.9); HRMS (FAB) *m/z* 216.0870 $(M + H)^+$ calcd for $C_9H_{14}O_5N$, 216.0872.

***β*-Allokainic acid norketone (11):** mp 211−212 °C dec; $[\alpha]_D$ −16.6° (*c* 0.350, H_2O) [lit.[21e] mp 213 °C dec; $[\alpha]_D$ −23.5° (*c* 1.0 H_2O)]; IR (Nujol) 2910, 1715, 1635, 1455, 1380, 1175 cm^{-1}; ^1H NMR (400 MHz, pD 3.0) δ 2.35 (3H, s), 2.57 (1H, dd, J = 9.3, 17.1), 2.62 (1H, dd, J = 5.9, 17.1), 3.17 (1H, dddd, J = 5.4, 5.9, 7.8, 9.3), 3.49 (1H, dt, J = 8.3, 5.4), 3.57 (1H, dd, J = 5.4, 12.2), 3.69 (1H, dd, J = 8.3, 12.2), 4.22 (1H, d, J = 7.8); HRMS (FAB) *m*/*z* 216.0888 $(M + H)^+$ calcd for $C_9H_{14}O_5N$, 216.0872.

α-Dihydrokainic acid (12): mp 277−279 °C dec; $[\alpha]_D$ −40.6° (*c* 0.33, H_2O) [lit.[21a] mp 278 °C dec; $[\alpha]_D$ −28.4° (*c* 5.0, H_2O)]; IR (Nujol) 3100, 2920, 1730, 1625, 1470, 1460, 1385, 1335, 1290, 1250, 1210, 1180, 885, 870 cm^{-1}; ^1H NMR (400 MHz, pD 3.1) δ 0.91 (3H, d, J = 6.5), 0.95 (3H, d, J = 6.5), 1.60 (1H, doublet of septet, J = 12.0, 6.5), 1.98 (1H, ddt, J = 5.5, 8.0, 12.0), 2.26 (1H, dd, J = 12.0, 16.0), 2.68 (1H, dd, J = 4.5, 12.0), 3.01 (1H, dddd, 1.0, 4.5, 5.5, 12.0), 3.08 (1H, t, J = 12.0), 3.65 (1H, dd, J = 8.0, 12.0), 4.14 (1H, d, J = 1.0); HRMS (FAB) *m*/*z* 216.1246 $(M + H)^+$ calcd for $C_{10}H_{18}O_4N$, 216.1236.

***β*-Dihydrokainic acid (13):** mp 232−234 °C dec; $[\alpha]_D$ +25.7° (*c* 0.14, H_2O) [lit.[21b] mp 230 °C dec; $[\alpha]_D$ +24.0° (*c* 5.0, H_2O)]; IR (Nujol) 3600, 3140, 2920, 1695, 1465, 1380, 1295, 1245, 1175, 1050, 935, 785, 735 cm^{-1}; ^1H NMR (400 MHz, pD 3.4) δ 0.92 (3H, d, J = 6.4), 0.96 (3H, d, J = 6.4), 1.65 (1H, doublet of septet, J = 11.0, 6.4), 2.21 (1H, dddd, J = 5.5, 8.3, 11.0, 12.0), 2.23 (1H, dd, J = 7.6, 16.8), 2.51 (1H, dd, J = 4.6, 16.8), 3.10 (1H, t, J = 12.0), 3.15 (1H, dddd, J = 4.6, 5.5, 5.9, 7.6), 3.53 (1H, dd, J = 8.3, 12.0), 4.23 (1H, d, J = 5.9); HRMS (FAB) *m*/*z* 216.1223 $(M + H)^+$ calcd for $C_{10}H_{18}O_4N$, 216.1236.

α-Dihydroallokainic acid (14): mp 251−254 °C dec; $[\alpha]_D$ −20.8° (*c* 0.24, H_2O) [lit.[21c] mp 249−250 °C dec; $[\alpha]_D$ −19.8° (*c* 3.02, H_2O)]; IR (Nujol) 2900, 1715, 1630, 1595, 1460, 1380, 1315, 1270, 1220, 955, 885 cm^{-1}; ^1H NMR (400 MHz, pD 3.4) δ 0.91 (3H, d, J = 6.8), 0.98 (3H, d, J = 6.8), 1.77 (1H, octet, J = 6.8), 2.09 (1H, dddd, J = 6.3, 6.8, 7.8, 9.3), 2.63 (1H, quintet, J = 6.3), 2.75 (2H, d, J = 6.3), 3.23 (1H, dd, J = 9.3, 12.0), 3.54 (1H, dd, J = 7.8, 12.0), 4.00 (1H, d, J = 6.3); HRMS (FAB) *m*/*z* 216.1226 $(M + H)^+$ calcd for $C_{10}H_{18}O_4N$, 216.1236.

***β*-Dihydroallokainic acid (15):** mp 220−222 °C dec; $[\alpha]_D$ +3.50° (*c* 0.20, H_2O) [lit.[21d] mp 220 °C dec; $[\alpha]_D$ +3.5° (*c* 1.0, H_2O)]; IR (Nujol) 2910, 1715, 1615, 1465, 1380 cm^{-1}; ^1H NMR (400 MHz, pD 3.4) δ 0.92 (3H, d, J = 6.8), 0.99 (3H, d, J = 6.8), 1.82 (1H, octet, J = 6.8), 2.27 (1H, dddd, J = 6.8, 7.5, 8.1, 9.0), 2.49 (1H, dd, J = 7.6, 16.6), 2.61 (1H, dd, J = 6.4, 16.6), 2.79 (1H, dddd, J = 6.4, 7.5, 7.6, 8.8), 3.14 (1H, dd, J = 9.0, 12.0), 3.62 (1H, dd, J = 8.1, 12.0), 4.27 (1H, d, J = 8.8); HRMS (FAB) *m*/*z* 216.1220 $(M + H)^+$ calcd for $C_{10}H_{18}O_4N$, 216.1236.

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