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On the Relationship between the Chemical Structure and the Cyclic AMP Phosphodiesterase Inhibitory Activity of Flavonoids as Studied by ^{13}C NMR

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The relationship between the chemical structure and the cyclic adenosine monophosphate phosphodiesterase inhibitory activity was studied regarding forty-eight flavones, thirty-five flavanones, and six isoflavones by using the ^{13}C NMR chemical shift averaged over all aromatic carbons in a compound. The average value of potent ^{13}C chemical shifts over the aromatic carbons active flavonoids (IC_{50} 0.6—20.0 $\times 10^{-5}$ M/L) are within the range from δ 133.5 to δ 135.7, but those of the inactive flavonoids and the weak active flavonoids are not within this range. This means that the averaged ^{13}C chemical shift becomes an "index" for testing the inhibitory activity. Further, the above relationship was proposed as a means for conveniently determining the inhibitory activity.

3′,5′-Cyclic adenosine monophosphate (cAMP) phosphodiesterase (PDE) inhibition can be used as a screening agent to detect physiologically active compounds.[1)] It is known that there is a close relationship between the inhibition of PDE in vitro among 158 therapeutic agents tested for PDE inhibition and the physiological activity in vivo.[2–6)] Moreover, many flavonoids have been identified as having a potent inhibitory activity on cAMP PDE.[7–10)] These results indicate that the PDE inhibitors might have a variety of physiological activities. However, it takes a long time to determine such physiological activities using the conventional method. Thus, it has been important to develop a simple method.

We studied the relationship between the inhibitory activity on cAMP PDE of flavonoids and the averaged ^{13}C NMR chemical shifts (δ_{av}) for all carbons in the ring structures of these compounds. We also clarified whether or not the averaged ^{13}C NMR chemical shift is useful for studying the cAMP PDE inhibitory activity and using its relationship as a simple method for determining physiological activities. The averaged ^{13}C NMR chemical shifts employed in this work reflect the electronic structure and electronic states of the molecules[11,12)] which may govern the mechanism of the inhibitory activity. As shown in the theory of ^{13}C shielding in aromatic hydrocarbons,[13)] the chemical shift δ of each carbon can be expressed as $\delta \propto 1/\Delta E \cdot Q$, where ΔE is the averaged excitation energy, and Q is a function of the electron density and bond order of a molecule.

The averaged excitation energy is assumed to be a constant for the aromatic compounds considered here.[11)] Thus, we expect that the averaged ^{13}C NMR chemical shift is a measure of the inhibitory activity.

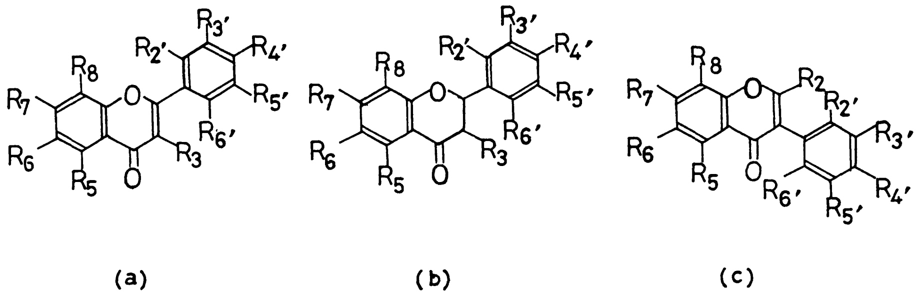
Experimental

Authentic samples were used in the tests for the evaluation of the inhibitory activity on cAMP phosphodiesterase which had been isolated or prepared during structural studies.[1,8,14)] (Tables 1, 2, and 3), (Fig. 1 (a), (b), and (c)). Cyclic AMP PDE inhibitory activity was assayed by the method described in a previous paper.[14)]

The ^{13}C NMR spectra were recorded with Fourier transform spectrometers (JEOL JNM FX-90 Q, JEOL JNM GX-270 and JEOL JNM GX-400) operating at 22.5, 67.5, and 100.4 MHz, respectively, at room temperature. Dimethyl-d_6 sulfoxide was used as a solvent. The concentration of the samples was 2—10% (w/v). The chemical shifts were expressed in ppm relative to tetramethylsilane (TMS).

Results and Discussion

The averaged ^{13}C NMR chemical shifts (δ_{av}) over all the ring carbons of flavonoids and 50% inhibitory concentrations (IC_{50})cAMP PDE inhibitory activities of these samples are shown in Tables 1, 2, and 3. The compounds listed in these tables can be divided into three groups: potent inhibitory groups (IC_{50}=0.6—20.0$\times 10^{-5}$ M/L and δ_{av}=133.5—135.7), weak inhibitory groups (IC_{50}=20.1—35.3$\times 10^{-5}$M/L and $\delta_{av} \leqq$ 133.5), and inactive groups ($IC_{50} \geqq$35.3$\times 10^{-5}$ M/L$\geqq$135.7). The relationship between the δ_{av} of the individual compound and the IC_{50} value in the cAMP PDE inhibition is shown in Fig. 2. As can be seen from this figure, it is found that there is a correlation between IC_{50} and δ_{av} in the range from 133.5 to 135.7 . This means that δ_{av} can be used to discern whether the



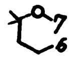
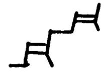
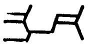
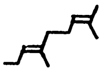
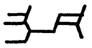
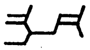
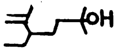
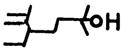
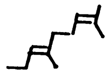
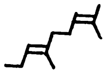
(a) (b) (c)

Fig. 1. (a) Flavone compounds. (b) Flavanone compounds. (c) Isoflavone compounds.

Table 1. cAMP PDE Inhibitory Activity and Averaged ^{13}C NMR Chemical Shifts ($\bar{\delta}$) of Flavones

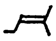
	Substituents											Averaged ^{13}C
No.	R_3	R_5	R_6	R_7	R_8	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$	$R_{6'}$	IC_{50} ($\times 10^{-5}$M/L)	($\bar{\delta}$)
1	H	H	H	H	H	H	H	H	H	H	13.2	133.7
2	H	OH	H	OMe	H	H	H	H	H	H	150.0	132.6
3	H	OH	OH	OH	H	H	H	H	H	H	7.6	133.5
4	H	OH	OMe	OH	H	H	H	H	H	H	5.7	134.6
5	H	OH	OH	Oglu	H	H	H	H	H	H	24.2	133.7
6	H	OH	H	OH	OH	H	H	H	H	H	7.2	133.7
7	H	OH	H	OH	OMe	H	H	H	H	H	15.0	134.7
8	H	OH	H	OMe	OMe	H	H	H	H	H	5.3	134.5
9	H	OMe	H	OH	OMe	H	H	H	H	H	7.2	134.2
10	H	OH	H	OAc	OMe	H	H	H	H	H	16.9	135.3
11	H	OAc	H	OAc	OMe	H	H	H	H	H	31.0	135.4
12	H	OMe	H	OAc	OMe	H	H	H	H	H	7.8	134.8
13	H	OH	H	OH	H	OH	H	H	H	H	14.2	133.7
14	H	OH	H	OH	H	H	H	OH	H	H	26.9	133.4
15	H	OH	H	OMe	H	H	H	OH	H	H	61.6	132.6
16	H	OH	H	OH	H	H	H	OMe	H	H	57.4	132.7
17	OH	OH	H	OH	H	H	H	OH	H	H	22.5	133.0
18	H	OH	OH	OH	H	H	H	OH	H	H	21.2	133.2
19	H	OH	OMe	OH	H	H	H	OH	H	H	4.2	134.2
20	H	OH	OMe	OMe	H	H	H	OH	H	H	11.8	134.3
21	H	OH	OMe	OMe	H	H	H	OMe	H	H	31.6	134.2
22	H	OH	H	OH	OMe	H	H	OH	H	H	6.5	134.4
23	H	OH	OMe	OMe	OH	H	H	H	H	H	8.3	135.7
24	H	OH	H	OH	H	H	OH	OH	H	H	20.8	133.3
25	H	OH	H	OH	H	H	OMe	OH	H	H	35.3	133.0
26	H	Oglu	H	OH	H	H	OH	OH	H	H	62.5	133.2
27	H	OH	H	Oglu	H	H	OH	OH	H	H	32.3	133.1
28	H	OH	H	OH	OMe	OH	H	H	H	H	0.6	134.6
29	H	OH	H	OH	OMe	OMe	H	H	H	H	6.7	134.7
30	H	OMe	H	OH	OMe	OMe	H	H	H	H	7.2	134.3
31	H	OH	H	OMe	OMe	OAc	H	H	H	H	5.5	135.7
32	H	OAc	H	OMe	OMe	OAc	H	H	H	H	>500.0	135.8
33	H	OH	H	OH	OMe	OH	H	H	OH	OMe	2.5	135.7
34	H	OH	OMe	OH	H	H	OH	OH	H	H	5.3	133.9
35	H	OH	OMe	OMe	H	H	OMe	OH	H	H	3.2	134.0
36	H	OH	H	OH	OMe	OH	H	H	H	OMe	16.9	134.7
37	H	OH	H	OMe	OMe	OH	H	H	H	OH	6.7	134.6
38	H	OH	H	OH	OMe	OMe	H	H	H	OMe	7.4	134.6
39	H	OH	H	OMe	OMe	OH	H	H	H	OMe	6.3	134.7
40	H	OH	H	OMe	OMe	OMe	H	H	H	OMe	16.5	134.6
41	H	OMe	OMe	OMe	OMe	H	H	OMe	H	H	28.2	136.7
42	OH	OH	H	OH	H	OH	H	H	H	OH	9.6	134.1
43	OH	OH	H	OH	H	OH	H	OH	H	H	16.3	133.1
44	OH	OH	H	OH	H	H	OH	OH	H	H	31.7	132.6
45	OMe	OMe	H	OMe	H	H	OMe	OMe	H	H	12.9	133.9
46	Orha	OH	H	OH	H	H	OH	OH	H	H	17.6	134.6
47	OH	OH	H	Oglu	H	H	OH	OH	H	H	13.9	133.5
48	OH	OH	H	OH	H	H	OH	Oglu	H	H	10.0	134.0

Table 2. cAMP PDE Inhibitory Activity and Averaged ^{13}C NMR Chemical Shifts ($\bar{\delta}$) of Flavanones

				Substituents							IC$_{50}$	Average ^{13}C($\bar{\delta}$)	
No.	R$_3$	R$_5$	R$_6$	R$_7$	R$_8$	R$_{2'}$	R$_{3'}$	R$_{4'}$	R$_{5'}$	R$_{6'}$	(×10^{-5}M/L)	(a)	(b)
49	H$_2$	H	H	OH		H	H	H	H	H	1.1	134.6	133.6
50	H$_2$	H	H	OMe		H	H	H	H	H	16.4	134.1	133.5
51	H$_2$	H			H	H	H	OH	H	H	4.5	135.9	134.6
52	H$_2$	H			H	H	H	OH	H	H	18.4	134.3	
53	H$_2$	H	H	OMe	Me	H	H	OMe	H	H	11.7	133.6	
54	H$_2$	OH	H	OMe		H	H	H	H	H	16.9	135.1	133.9
55	H$_2$	OH	H	OH	H	H	H	OH	H	H	109.0	136.1	
56	H$_2$	OH		OH	H	H	H	OH	H	H	6.0	136.5	135.3
57	H$_2$	OH	H	OH		H	H	OH	H	H	2.8	136.4	134.7
58	H$_2$	OH	H	OH		H		OH	H	H	2.4	136.5	134.4
59	H$_2$	OH	H	OH		H	H	OH	H	H	2.7	136.4	134.7
60	H$_2$	OH	OH	OH	H	H	H	H	H	H	5.3	135.4	
61	H$_2$	OH	OMe	OH	H	H	H	H	H	H	>500.0	136.1	
62	H$_2$	OH	Me	OMe	H	H	H	OMe	H	H	6.2	134.3	
63	H$_2$	OH	OH	OgluAOMe	H	H	H	OH	H	H	3.8	135.1	
64	H$_2$	OH	H	OgluAOMe	OH	H	H	OH	H	H	3.4	135.2	
65	H$_2$	OH	H	OMe	Me	H	H	OMe	H	H	8.1	133.9	
66	H$_2$	OH	H	OH	OMe	OH	H	H	H	H	14.1	135.7	
67	H$_2$	OH	H	OH	OMe	H	H	OH	H	H	32.4	136.2	
68	H$_2$	OH	H	OH	OMe	OMe	H	H	H	H	6.2	136.0	
69	H$_2$	OMe	H	OH	OMe	OMe	H	H	H	H	20.5	135.2	
70	H$_2$	OH	H	OMe	OMe	OH	H	H	OH	H	9.2	135.6	
71	H$_2$	OH	H	OMe	OMe	OH	H	H	H	OMe	9.8	135.7	
72	H$_2$	OH	OMe	OMe	H	OH	H	H	H	OMe	13.4	135.7	
73	H$_2$	OH	H	OH		OH	H	H	H	H	1.4	136.3	134.6
74	H$_2$	OH	H	OH		OH	H	OH	H	H	4.6	136.0	134.3
75	H$_2$	OH	H	OH		OH	H	OH	H	H	3.9	135.9	134.3
76	H$_2$	OH	H	OH		OMe	H	OH	H	H	5.7	135.2	133.7
77	H$_2$	OMe	H	OH		OH	H	OH	H	H	2.5	135.4	133.9
78	H$_2$	OMe	H	OH		OH	H	OH	H	H	6.3	135.4	134.7
79	H$_2$	OH		OH		OH	H	OH	H	H	4.0	136.4	134.4
80	H$_2$	OH		OH		OH	H	OH	H	H	3.1	136.5	134.8
81	OH	OMe	H	OH		OH	H	OH	H	H	17.2	135.6	134.9
82	H$_2$	OH		OH	H	OH	H	OH	H	H	3.1	136.0	134.3
83	H$_2$	OH	H	OH		OH	H	OH	H	OH	5.0	136.1	134.3

compounds being considered are potent inhibitors or not. Thirty flavones compounds **1**, **3**, **4**, **6**, **7**, **8**, **10**, **12**, **13**, **19**, **20**, **22**, **23**, **28**, **29**, **30**, **31**, **33**, **34**, **35**, **36**, **37**, **38**, **39**, **40**, **42**, **45**, **46**, **47**, and **48** have potent cAMP PDE inhibitory activity,[8] and their averaged chemical shifts are within the range δ 133.5 to 135.7. The δ_{av} values for the remaining eleven compounds **2**, **14**, **15**, **16**, **17**, **18**, **24**, **25**, **26**, **27**, and **44** are smaller than 133.5, while those for compounds **32** and **41** are larger than 135.7. The thirteen compounds, except for **32**

Table 3. cAMP PDE Inhibitory Activity and Averaged ^{13}C NMR Chemical Shifts ($\bar{\delta}$) of Isoflavones

No.	Substituents R_5	R_6	R_7	R_8	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$	$R_{6'}$	IC_{50} ($\times 10^{-5}$M/L)	Averaged ^{13}C ($\bar{\delta}$) (a)	(b)
84	H	H	OH	H	H	H	OH	H	H	28.3	133.0	
85	H	H	OH	H	H	H	OMe	H	H	49.6	132.8	
86	H	H	OH	H	H	OH	OMe	H	H	17.3	133.5	
87	OH	H	OH	H	H	H	OH	H	H	12.1	133.8	
88	OH		OH		H	H	OH	H	H	20.3	134.8	133.1
89	OH	OMe	OH	H	H	OH	OMe	OMe	H	1.1	134.7	

a) The δ_{av} of the individual compounds having no prenyl groups. b) The δ_{av} of the individual compounds having prenyl groups or having no prenyl groups and isoflavones.

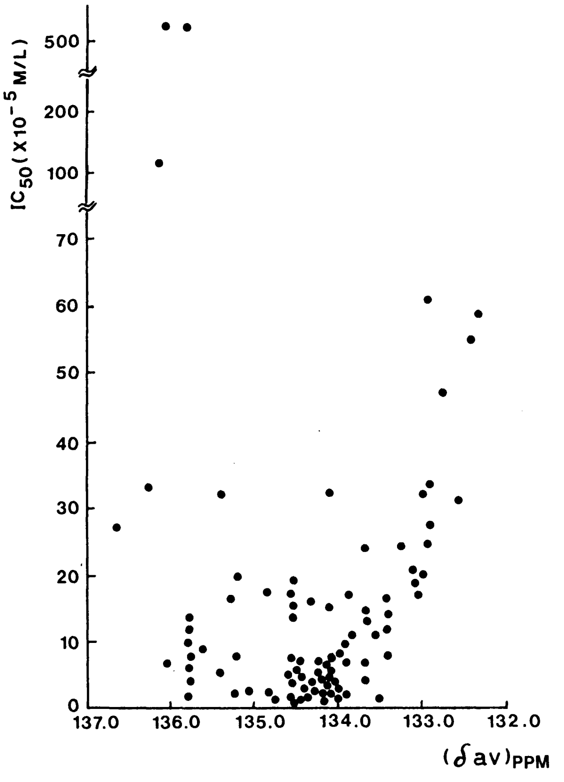


Fig. 2. The relationship between the averaged ^{13}C NMR chemical shifts (δ_{av}) of flavones and their cAMP PDE inhibitory activity IC_{50}.

and **41**, have a large IC_{50} value (>20.1) and, thus, inactive property. The δ_{av} value of **43** is slightly lower than 133.5, suggesting that this compound has a strong cAMP PDE inhibitory activity.

The values of δ_{av} for **5**, **11**, and **21** are within the range 133.5—135.7; thus, these compounds can be expected to be in the potent inhibitory group. However, the values of IC_{50} for **5**, **11**, and **21** are not contained in the potent inhibitory group. This means that it is not easy to exactly determine the borderline region.

Next, we consider the effect of the substituent group of the compounds on the relationship between the cAMP PDE inhibitory activity and δ_{av}. When the hydroxyl group or methoxyl group is substituted at the C-5, 7 or 4′-positions on an aromatic ring of compounds **14**, **15**, and **16**, their cAMP PDE inhibitory activity is detected slightly or not and the values of δ_{av} are smaller than 133.5. When the methoxyl group is substituted at the C-6 position on an aromatic ring in compounds **4**, **19**, **20**, **23**, **34**, and **35**, the increase in cAMP PDE inhibitory activity can be recognized[8] and the δ_{av} values are within the range 133.5—135.7. When the hydroxyl group, methoxyl group or *o*-glucosyl group is substituted at the C-5, 7, 3′ or 4′-positions on an aromatic ring of compounds **24**, **25**, **26**, and **27**, the cAMP PDE inhibitory activity is detected slightly or not and the values of δ_{av} are smaller than 133.5. However, when the methoxyl group or *o*-rhamnosyl group is substituted at the C-3 position on the aromatic ring of compounds **45** and **46**, the increase in cAMP PDE inhibitory activity can be recognized and the δ_{av} values are within the range 133.5—135.7. Eleven flavanones compounds **51**, **56**, **57**, **58**, **59**, **73**, **74**, **75**, **79**, **82**, and **83** have potent cAMP PDE inhibitory activity.[9] However, as can be seen from Table 2, the δ_{av} value of compounds having no prenyl, geranyl and lavandulyl group **51**, **56**, **57**, **58**, **59**, **79**, and **80**, are within the range 135.9—136.5, beyond the range 133.5—135.7.

On the other hand, in the case of having a prenyl group, it has been found that the value of δ_{av} for compounds **49**, **50**, **54**, **76**, **77**, **78**, and **81** are within the range 133.5—135.7, having a prenyl group.

It is considered that the prenyl group is a factor for having the cAMP PDE inhibitory activity, from the result of using the averaged value of the ^{13}C NMR chemical shift.

Flavanones derivatives[9] having no prenyl groups **52**, **53**, **60**, **62**, **63**, **64**, **65**, **66**, **69**, **70**, **71**, and **72** have a potent cAMP PDE inhibitory activity, and the δ_{av} values are within the range 133.5 to 135.7, as well as flavones. Compounds **55**, **61**, and **67** can be expected to be inactive groups or weak inhibitory group; the values of δ_{av} are larger than 135.7. Similary, concerning isoflavone derivatives, compounds **86**, **87**, **88**, and **89** showed potent cAMP PDE inhibitory activity.

The δ_{av} values of compounds **84** and **85** are smaller than 133.5. Therefore, these can be expected to be a weak inhibitory group or an inactive group.

Finally, we conclude the following. The cAMP PDE inhibitory activity for flavonoids can be examined from the averaged ^{13}C NMR chemical shift over all ring carbons. Further, it can be suggested that it is very useful to use this approach for drug design.

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