Inhibition of Adenosine 3′,5′-Cyclic Monophosphate Phosphodiesterase by Flavonoids. III[1)]

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Sixty-one flavanones, twenty-six isoflavones and eight other flavonoids, obtained from *Sophora tomentosa*, *S. flavescens*, *Scutellaria baicalensis* and other medicinal plants or synthesized, were tested for their inhibitory activity against adenosine 3′,5′-cyclic monophosphate (cAMP) phosphodiesterase from beef heart. The structure–activity relationships were investigated.

Keywords isoflavone; cAMP phosphodiesterase; inhibitor; structure–activity relationship; flavanone

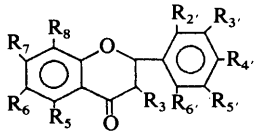
The adenosine 3′,5′-cyclic monophosphate (cAMP) phosphodiesterase inhibition test provides a useful tool for the screening of biologically active compounds contained in medicinal plants. We have identified flavonoids contained in various medicinal plants as cAMP phosphodiesterase inhibitors.[2-5)] The present paper deals with the inhibition of cAMP phosphodiesterase by flavanones, isoflavones and other flavonoids, most of which had been isolated from *Sophora tomentosa*, *Sophora flavescens* and *Scutellaria baicalensis*. The structure–activity relationships of these compounds and some related flavonoids were investigated.

Results and Discussion

In order to study structure–activity relationships, a large number of flavanones and isoflavones were screened for their inhibitory activity on cAMP phosphodiesterase by measuring the concentration (IC_{50}) required to give 50% inhibition of this enzyme activity. The test compounds were sixty-one flavanones, twenty-six isoflavones and eight flavonoids isolated from various medicinal plants or synthesized. The results are summarized in Tables I and II.

The structure–activity relationships can be summarized as follows. (1) Flavanones (**1**—**61**, Table I). (a) Intro-

TABLE I. Inhibitory Activity of Flavanones on cAMP Phosphodiesterase



No.	Substituent										IC_{50} ($\times 10^{-5}$ M)	Source & reference
	R_3	R_5	R_6	R_7	R_8	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$	$R_{6'}$		
1	H_2	H	H	OH	H	H	H	H	H	H	>500	Syn.[6)]
2	H_2	H	H	$-OCH_2CH=C(CH_3)_2$	H	H	H	H	H	H	29.1	Syn.[6)]
3	H_2	H	H	OH	Pr	H	H	H	H	H	1.1	Syn.[6)]
4	H_2	H	H	$-OC(CH_3)_2CH=CH-$		H	H	H	H	H	12.6	L.X.[7)]
5	H_2	H	H	$-OC(CH_3)_2CH_2CH_2-$		H	H	H	H	H	7.2	Syn.[8)]
6	H_2	H	H	OMe	Pr	H	H	H	H	H	16.4	L.v.[6)]
7	H_2	H	H	OMe	$CH_2=CHCH=CH(CH_3)$	H	H	H	H	H	17.9	T.v.[9)]
8	H_2	H	H	OH	H	H	H	OH	H	H	46.4	Syn.[10)]
9	H_2	H	H	OH	Pr	H	H	OH	H	H	27.0	L.n.[11)]
10	H_2	H	Pr	OMe	H	H	H	OH	H	H	347	Syn.[12)]
11	H_2	H	Me	OMe	H	H	H	OMe	H	H	10.1	Syn.[13)]
12	H_2	H	H	OMe	Me	H	H	OMe	H	H	11.7	Syn.[13)]
13	H_2	H	$-CH=CH(CH_3)_2CO-$		H	H	H	OH	H	H	4.5	Syn.[12)]
14	H_2	H	$-CH_2CH_2(CH_3)_2CO-$		H	H	H	H	H	H	18.4	Syn.[14)]
15	H_2	OH	H	OMe	H	H	H	H	H	H	99.1	Syn.[15)]
16	H_2	OH	H	OMe	Pr	H	H	H	H	H	16.9	T.sp.[9)]
17	H_2	OH	H	OH	H	H	H	OH	H	H	109	S.t.[16)]
18	H_2	OH	H	OMe	H	H	H	OH	H	H	43.3	H.s.[17)]
19	H_2	OH	Pr	OH	H	H	H	OH	H	H	6.0	Syn.[16)]
20	H_2	OH	H	OH	Pr	H	H	OH	H	H	2.8	S.t.[16)]
21	H_2	OH	H	OH	Pr	H	Pr	OH	H	H	2.4	E.j.[16)]
22	H_2	OH	H	OH	Ger	H	H	OH	H	H	2.7	S.t.[16)]
23	H_2	OH	OH	OH	H	H	H	H	H	H	5.3	S.s.[18)]
24	H_2	OH	OMe	OH	H	H	H	H	H	H	>500	S.b.[19)]
25	H_2	OH	Me	OMe	H	H	H	OMe	H	H	6.2	Syn.[20)]
26	H_2	OH	OH	OgluAOMe	H	H	H	OH	H	H	3.8	S.b.
27	H_2	OH	OMe	OgluAOMe	H	H	H	OMe	H	H	7.0	Syn.
28	H_2	OMe	OMe	OgluAOMe	H	H	H	OMe	H	H	3.9	Syn.[21)]

TABLE I. (continued)

No.	Substituent										IC_{50} ($\times 10^{-5}$ M)	Source & reference
	R_3	R_5	R_6	R_7	R_8	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$	$R_{6'}$		
29	H_2	OH	H	OgluAOMe	OH	H	H	OH	H	H	3.4	S.b.
30	H_2	OH	H	OMe	Me	H	H	OMe	H	H	8.1	Syn.[20]
31	H_2	OH	H	OH	H	OH	H	OH	H	H	37.7	C.t.[22]
32	H_2	OH	H	OMe	H	OMe	H	OMe	H	H	46.4	C.t.[22]
33	H_2	OMe	H	OMe	H	OMe	H	OMe	H	H	25.9	C.t.[22]
34	H_2	OH	H	OH	OMe	OH	H	H	H	H	14.1	S.i.[23]
35	H_2	OH	H	OH	OMe	H	H	OH	H	H	32.4	S.r.[24]
36	H_2	OH	H	OH	OMe	OMe	H	H	H	H	6.2	S.d.[25]
37	H_2	OMe	H	OH	OMe	OMe	H	H	H	H	20.5	S.d.[25]
38	H_2	H	OMe	OMe	H	H	OMe	OMe	H	H	10.4	Syn.[15]
39	OH	OH	H	OH	H	H	OH	OH	H	H	32.3	Syn.[15]
40	OH	OH	H	OH	H	OMe	H	$-OC(CH_3)_2CH=CH-$		H	3.8	S.t.[26]
41	H_2	OH	H	OMe	OMe	OH	H	H	OH	H	9.2	S.i.[23]
42	H_2	OH	H	OMe	OMe	OH	H	H	H	OMe	9.8	S.d.[25]
43	H_2	OH	H	OMe	OMe	OgluA	H	H	H	OMe	26.2	S.i.[23]
44	H_2	OH	H	OMe	OMe	OgluAOMe	H	H	H	OMe	21.6	S.i.
45	H_2	OH	OMe	OMe	H	OH	H	H	H	OMe	13.4	S.d.[25]
46	O-rha	OH	H	OH	H	H	OH	OH	H	H	38.8	A.t.[27]
47*	$-C_{8'}$	OH	H	OH	H	H	H	OH	H	H		
	H	OH	H	OH	$-C_8$	H	OH	OH	H	H	0.5	R.sp[28]
48	H_2	OH	H	OH	Lav	OH	H	H	H	H	1.4	S.f.[4]
49	H_2	OMe	H	OH	Pr	H	H	OH	H	H	>500	S.f.[4]
50	H_2	OH	H	OH	Ger	OH	H	OH	H	H	4.6	S.t.[29]
51	H_2	OH	H	OH	Lav	OH	H	OH	H	H	3.9	S.f.[4]
52	H_2	OH	H	OH	Lav	OMe	H	OH	H	H	5.7	S.f.[4]
53	H_2	OMe	H	OH	Lav	OH	H	OH	H	H	2.5	S.f.[4]
54	H_2	OMe	H	OH	Lav-OH	OH	H	OH	H	H	6.3	S.f.[4]
55	H_2	OH	H	OMe	Lav	OMe	H	OMe	H	H	>500	Syn.[4]
56	H_2	OMe	H	OMe	Lav	OMe	H	OMe	H	H	>500	Syn.[4]
57	H_2	OH	Pr	OH	Pr	OH	H	OH	H	H	4.0	S.f.[4]
58	H_2	OH	Pr	OH	Lav	OH	H	OH	H	H	3.1	S.f.[4]
59	OH	OMe	H	OH	Lav-OH	OH	H	OH	H	H	17.2	S.f.[4]
60	H_2	OH	Ger	OH	H	OH	H	OH	H	OH	3.1	S.t.[30]
61	H_2	OH	H	OH	Ger	OH	H	OH	H	OH	5.0	S.t.[31]

Abbreviations: A.t., *Astilbe thunbergii*; C.t., *Chlorophora tinctoria*; E.j., *Euchresta japonica*; gluA, glucuronic acid; gluAOMe, glucuronic acid methyl ester; H.s., *Hyptis salzhanii*; L.n., *Lonchocarpus neuroscapha*; L.v., *Lonchocarpus violenceus*; L.x., *Lonchocarpus xuul*; rha, rhamnose; R.sp, *Rheeoia sp.*; S.b., *Scutellaria baicalensis*; S.d., *Scutellaria discolor*; S.f., *Sophora flavescens*; S.i., *Scutellaria indica*; S.r., *Scutellaria rivularis*; S.s., *Scutellaria scandens*; S.t., *Sophora tomentosa*; Syn., synthesis; T.sp, *Tephrosia sp.*; T.v., *Tephroshia vagelii*. **47***, dimeric flavanone.

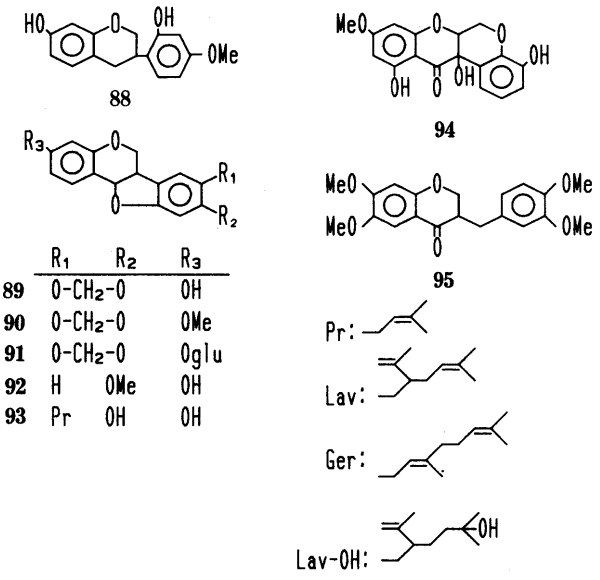


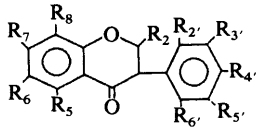
Chart 1

duction of a prenyl or geranyl group into 7-hydroxyflavanones (**3** and **9**) or 4′,5,7-trihydroxyflavanones (**19**—**22**) caused a remarkable increase of inhibitory activity on cAMP phosphodiesterase. (b) In the case of 7-*O*-methylflavanones (**6**, **7**, **10** and **16**), the degree of increase of inhibitory activity on cAMP phosphodiesterase by introduction of a prenyl group is lower than in the cases of 7-hydroxy- and 4′,5,7-trihydroxyflavanones. (c) The flavanones having prenyl, geranyl or lavandulyl groups (**3**, **6**, **9**, **10**, **16**, **19**—**22** and **48**—**61**) showed greater inhibitory activity than the corresponding flavanones (**1**, **8**, **15**, **17** and **31**) irrespective of the location and number of the substituents. (d) The flavanones having a glucuronic acid methylate group in the A-ring (**26**—**29**) exhibited potent inhibitory activities, but the effects of **43** and **44** in which the glucuronic acid methylate group is located at the 2′ position, are not so potent. (2) Isoflavones (**62**—**87**, Table II). (a) Polyfunctional isoflavones (**78**—**82**) exhibit comparatively high inhibitory activity compared with less highly functionalized ones. (b) The potencies of isoflavones (**73**, **83**, **84** and **87**) having prenyl, O-prenyl or hydrated prenyl groups are not so high as those of flavanones. (3) Other flavonoid (**88**—**95**, Table II). Almost all these flavonoids exhibited relatively weak activity except for prenylated pterocarpane (**93**) and homoisoflavone (**95**).

Experimental

Assay Method for cAMP Phosphodiesterase-Inhibitory Activity The

TABLE II. Inhibition of Cyclic AMP Phosphodiesterase by Isoflavones and Other Flavonoids



No.	Substituent										IC_{50} ($\times 10^{-5}$ M)	Source & reference
	R_2	R_5	R_6	R_7	R_8	$R_{2'}$	$R_{3'}$	$R_{4'}$	$R_{5'}$	$R_{6'}$		
62	H	H	H	OH	H	H	H	OH	H	H	28.3	P.p.[2]
63	H	H	H	OH	H	H	H	OMe	H	H	49.6	P.p.[2]
64	H	H	OH	OMe	H	H	H	OH	H	H	44.5	Syn.[15]
65	H	H	H	Oglu	H	H	H	OH	H	H	28.4	P.p.[2]
66	H	H	H	OH	H	OH	H	OMe	H	H	44.5	Syn.[15]
67	H	H	H	OH	H	OMe	H	OMe	H	H	6.6	Syn.[15]
68	H	H	H	OMe	H	OMe	H	OMe	H	H	5.5	Syn.[15]
69	H	H	H	OH	H	H	OH	OMe	H	H	17.3	S.t.[30]
70	H	H	H	OMe	H	H	OMe	OMe	H	H	7.2	Syn.[15]
71	H	OH	H	OH	H	H	H	OH	H	H	12.1	P.p.[2]
72	H	OH	H	OH	H	H	H	OMe	H	H	69.5	Syn.[15]
73	H	OH	Pr	OH	Pr	H	H	OH	H	H	20.3	E.j.[32]
74	H	H	OMe	OH	H	H	H	OMe	H	H	15.4	Syn.[15]
75	H	H	OMe	OMe	H	H	H	OMe	H	H	16.2	Syn.[15]
76	H	H	OMe	OMe	H	H	OMe	OMe	H	H	22.1	Syn.[15]
77	H	OH	H	OH	H	H	OH	OH	H	H	15.6	P.p.[2]
78	H	OH	OMe	OH	H	H	OH	OMe	H	H	9.7	P.p.[2]
79	H	OH	OMe	OH	H	H	OH	OMe	OMe	H	1.1	P.p.[2]
80	H	OAc	OMe	OAc	H	H	OAc	OMe	OMe	H	10.0	P.p.[2]
81	H	OH	$-O-CH_2-O-$		H	H	OMe	OMe	OMe	H	28.8	P.p.[2]
82	H	OMe	$-O-CH_2-O-$		H	H	OMe	OMe	OMe	H	0.4	P.p.[2]
83	H	OH	$-CH=CHC(CH_3)_2O-$		H	H	H	OH	H	H	58.4	M.t.[33]
84	H	OMe	$-CH=CHC(CH_3)_2O-$		H	H	$-O-CH_2-O-$		H	H	>500	D.g.[34]
85	$-O-$	H	H	H	H	H	H	H	H	H	46.2	Syn.[35]
86	H	OH	H	OH	H	H	OH	OMe	H	H	17.8	S.t.[29]
87	H_2	OH	$-CH_2CH_2CH(CH_3)_2$	OH	H	OMe	$-CH_2CH_2CH(CH_3)_2$	OH	H	H	72.9	S.t.[36]
88											32.3	Syn.[15]
89											63.2	Syn.[15]
90											143	Syn.[15]
91											231	S.s.[37]
92											23.5	Syn.[15]
93											8.7	S.f.[38]
94											20.1	B.c.[39]
95											9.7	Syn.[15]

Abbreviations: B.c., *Boerhaavia coccinea*; E.j., *Euchresta japonica*; glu, glucose; P.p., previous paper; S.f., *Sophora franchetiana*; S.s., *Sophora subprostrata*; S.t., *Sophora tomentosa*; Syn., synthesis.

liquid scintillation counter used was an Aloka LSC-903. Samples were tested for inhibition of cAMP phosphodiesterase activity in duplicate by the method described in the previous paper.[5]

Enzymes and Chemicals Beef heart phosphodiesterase was purchased from Boehringer. Snake venom nucleotidase and cAMP were obtained from Sigma, and [^{3}H] cAMP from the Radiochemical Centre. Papaverine, a reference inhibitor, was purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo).

26: **26** was isolated from the leaves of *Scutellaria baicalensis* as an artifact, mp 213 °C (dec.), pale yellow needles, $[\alpha]_D^{24}$ −102° (c=0.08, MeOH). Ultraviolet (UV) λ_{max}^{MeOH} nm: 248 sh, 286, 362. Infrared (IR) ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1742 (ester), 1660 (conjugated CO), 1600 (arom. C=C). Circular dichroism (CD): $[\theta]^{24}$ (nm) +1840 (335), −2780 (284). Proton nuclear magnetic resonance (^{1}H-NMR) dimethyl sulfoxide (DMSO-d_6): 3.40 (3H, s, Me-ester), 11.85 (1H, s, 5-OH). The identity of **26** was confirmed by direct comparison with the methyl ester which was derived from (25*S*)-4′,5,6,7-tetrahydroxyflavanone 7-*O*-β-D-glucuronopyranoside[21] by treatment with 5% HCl–MeOH over-night.

27: **27** was prepared by treatment of (25*S*)-4′,5,6,7-tetrahydroxyflavanone 7-*O*-β-D-glucuronopyranoside[21] with diazomethane, mp 211—212 °C (dec.), colorless needles. ^{1}H-NMR (DMSO-d_6): 3.39 (3H, s, Me-ester), 3.69, 3.77 (each 3H, s, OMe×2), 11.97 (1H, s, 5-OH).

29: **29** was isolated from the leaves of *Scutellaria baicalensis* as an artifact, mp 230 °C (dec.), pale yellow needles. $[\alpha]_D^{24}$ −120 °C (c=0.08, MeOH). UV λ_{max}^{MeOH} nm: 243 sh, 285, 365. IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1736 (ester), 1650 (conjugated CO), 1600 (arom. C=C). CD $[\theta]^{24}$ (nm): −45100 (284), +1920 (311). ^{1}H-NMR (DMSO-d_6): 3.39 (3H, s, Me-ester), 11.70 (1H, s, 5-OH). **29** was confirmed by direct comparison with the methyl ester which was derived from (25*S*)-4′,5,7,8-tetrahydroxyflavanone 7-*O*-β-D-glucurono pyranoside[21] by treatment with 5% HCl–MeOH.

44: **44** was isolated from *Scutellaria indica* as an artifact, mp 214—216 °C (dec), colorless needles. ^{1}H-NMR (DMSO-d_6): 3.39 (3H, s, Me-ester), 3.68, 3.80. 3.85 (each 3H, s, MeO×3), 12.17 (1H, s, 5-OH).

Authentic Flavanones, Isoflavones and Other Flavonoids The authentic samples used for the tests of inhibitory assay on cAMP phosphodiesterase were isolated or prepared during structural studies (Tables I and II).

References and Notes

1) A part of this study was presented at the 107th Annual Meeting of the Pharmaceutical Society of Japan, Kyoto, April 1987. This paper forms Part XVI of "Inhibitors of cAMP Phosphodiesterase in Medicinal Plants." Part XV: T. Ohmoto, T. Nikaido, K. Koike, K. Kohda, and U. Sankawa, *Chem. Pharm. Bull.*, **36**, 4588 (1988).
2) T. Nikaido, T. Ohmoto, U. Sankawa, T. Hamanaka, and K. Totsuka, *Planta Medica*, **46**, 162 (1982).
3) T. Nikaido, T. Ohmoto, T. Nomura, T. Fukai, and U. Sankawa, *Chem. Pharm. Bull.*, **32**, 4929 (1984).
4) T. Ohmoto, R. Aikawa, T. Nikaido, U. Sankawa, W. L. Jun, A. Ueno, and S. Fukushima, *Chem. Pharm. Bull.*, **34**, 2094 (1986).
5) T. Nikaido, T. Ohmoto, U. Sankawa, T. Tomimori, Y. Miyaichi, and

Y. Imoto, *Chem. Pharm. Bull.*, **36**, 654 (1988).
6) O. G. D. Lima, G. B. M. Bettolo, J. F. D. Mello, F. D. Monache, J. S. D. B. Coelho, F. D. D. A. Lyra, and M. M. F. D. Albuqerque, *Gazz. Chim. Ital.*, **103**, 771 (1973).
7) F. D. Monache, L. E. C. Suarez, and G. B. M. Bettolo, *Phytochemistry*, **17**, 1812 (1978).
8) O. G. D. Lima, G. B. M. Bettolo, J. F. D. Mello, F. D. Monache, J. S. D. B. Coelho, F. D. A. Lyra, and M. M. F. D. Albuquerque, *Gazzetta Chim. Ital.*, **103**, 771 (1973).
9) L. E. C. Suarez, F. D. Monache, G. B. M. Bettolo, and F. Menichini, *IL. Farmaco. Ed. Sci.*, **35**, 795 (1980).
10) F. Ferrari, B. Botta, R. A. D. Lima, and G. M. Maciei, *Revista do Instituto de Antibioticos, Recife*, **21**, 205 (1982).
11) O. G. D. Lima, J. F. D. Mello, J. S. D. B. Coelho, F. D. D. A. Lyra, M. Machado, F. D. Albuquerque, G. B. M. Bettolo, G. D. Monache, and F. D. Monache, *IL. Farmaco. Ed. Sic.*, **30**, 326 (1975).
12) A. Lupi, G. D. Monache, F. D. Monache, G. B. M. Bettolo, O. G. D. Lima, and J. F. D. Mello, *IL. Farmaco Ed. Sci.*, **32**, 261 (1977).
13) S. Matsuura, *Yakugaku Zasshi*, **77**, 298 (1957).
14) F. D. Monache, O. G. D. Lima, J. F. D. Mello, G. D. Monache, and G. B. M. Bettolo, *Gazzetta Chim. Ital.*, **103**, 779 (1973).
15) Synthesis of flavonoids by H. Wagner and L. Farkas, in "*The Flavonoids*," ed. by J. B. Harborne, T. J. Mabry, and H. Mabry, Chapman and Hall, London, 1975, pp. 127—213 and references cited therein.
16) Y. Shirataki, I. Yokoe, M. Endo, and M. Komatsu, *Chem. Pharm. Bull.*, **33**, 444 (1985).
17) F. Ferrari, in preparation.
18) Y. Miyaichi, Y. Imoto, T. Tomimori, and T. Namba, *Chem. Pharm. Bull.*, **36**, 2371 (1988).
19) S. Takagi, M. Yamamoto, and K. Inoue, *Yakugaku Zasshi*, **100**, 1220 (1980).
20) S. Matsuura, *Yakugaku Zasshi*, **77**, 302 (1957).
21) Y. Miyaichi, Y. Imoto, H. Saida, and T. Tomimori, *Syoyakugaku Zasshi*, **42**, 216 (1988).
22) F. D. Monache, in preparation.
23) Y. Miyaichi, Y. Imoto, T. Tomimori, and C.-C. Lin, *Chem. Pharm. Bull.*, **35**, 3720 (1987).
24) T. Tomimori, Y. Miyaichi, Y. Imoto, and H. Kizu, *Shoyakugaku Zasshi*, **40**, 432 (1986).
25) T. Tomimori, Y. Miyaichi, Y. Imoto, H. Kizu, and T. Namba, *Chem. Pharm. Bull.*, **33**, 4457 (1985).
26) F. D. Monache, G. D. Monache, G. B. M. Bettolo, M. M. F. D. Albuquerque, J. F. D. Mello, and O. G. D. Lima, *Gazzetta Chim. Ital.*, **106**, 935 (1976).
27) H. Shimada, T. Sawada, and S. Fukuda, *Yakugaku Zasshi*, **72**, 578 (1952).
28) B. Botta, M. M. M. Quahe, G. D. Monache, and F. D. Monache, *J. Nat. Prod.*, **47**, 1053 (1984).
29) I. Yokoe, M. Endo, Y. Shirataki, and M. Komatsu, Abstracts of Papers, 103rd Annual Meeting of the Pharmaceutical Society of Japan, 1983, p. 220.
30) I. Yokoe, M. Endo, Y. Shirataki, and M. Komatsu, Abstracts of Papers, 30th Annual Meeting of the Japanese Society of Pharmacognosy, 1983, p. 79.
31) I. Yokoe, M. Endo, Y. Shirataki, and M. Komatsu, Abstracts of Papers, 104th Annual Maeeting of the Pharmaceutical Society of Japan, 1984, p. 180.
32) Y. Shirataki, A. Manaka, I. Yokoe, and M. Komatsu, *Phytochemistry*, **21**, 2959 (1982).
33) E. M. Olivares, W. Lwande, F. D. Monache, and G. B. M. Bettolo, *Phytochemistry*, **21**, 1763 (1982).
34) F. D. Monache, G. C. Valera, D. S. D. Zapata, and G. B. M. Bettolo, *Gazzetta Chim. Ital.*, **107**, 403 (1977).
35) J. A. Donnelly, J. R. Keegan, and K. Quigley, *Tetrahedron*, **36**, 1671 (1980).
36) *a*) G. D. Monache, F. D. Monache, G. B. M. Bettolo, M. M. F. D. Albuquerque, J. F. D. Mello, and O. G. D. Lima, *Gazzetta Chim. Ital.*, **107**, 189 (1977); *b*) M. Komatsu, I. Yokoe, and Y. Shirataki, *Chem. Pharm. Bull.*, **26**, 3863 (1978).
37) M. Komatsu, I. Yokoe, Y. Shirataki, and J. Chen, *Phytochemistry*, **15**, 1089 (1976).
38) M. Komatsu, I. Yokoe, and Y. Shirataki, *Chem. Pharm. Bull.*, **29**, 532 (1981).
39) I. Messana, F. Ferrari, and A. E. G. Santana, *Phytochemistry*, **25**, 2688 (1986).