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Synthesis of 3-Substituted Pyrazolo[1,5-*a*]pyridine Derivatives with Inhibitory Activity on Platelet Aggregation. I

KATSUYA AWANO,* SEIGO SUZUE and MITSURU SEGAWA

Central Research Laboratories, Kyorin Pharmaceutical Co., Ltd., Nogi-machi, Shimotsuga-gun, Tochigi 329-01, Japan

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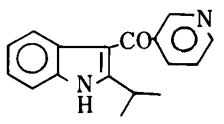
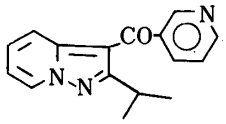
3-Nicotinoylpyrazolo[1,5-*a*]pyridines were synthesized by the reaction of 3-unsubstituted pyrazolo[1,5-*a*]pyridines with nicotinoyl chloride hydrochloride. Tetrahydronicotinoyl derivatives were obtained by hydrogenation of the nicotinoyl derivatives. Furthermore, *N*-substituted derivatives were synthesized by the reaction of the tetrahydronicotinoyl derivatives with alkylating reagents or isocyanates. These pyrazolo[1,5-*a*]pyridines were tested for inhibitory activity on arachidonic acid induced platelet aggregation *in vitro* and *ex vivo*. Some of these compounds showed higher inhibitory activity than aspirin. Among them, 2-methyl-3-(1,4,5,6-tetrahydronicotinoyl)pyrazolo[1,5-*a*]pyridine was found to be the most active compound.

Keywords——3-substituted pyrazolo[1,5-*a*]pyridine; tetrahydronicotinoyl derivative; *N*-substituted derivative; platelet aggregation inhibitory activity

It is generally accepted that platelets and/or products of arachidonic acid metabolism play an important role in the etiology of thrombotic diseases and arteriosclerosis. Therefore, much work has been done to develop effective antiaggregants. During synthetic studies on pyrazolo[1,5-*a*]pyridines, we noted the structural similarity of compound **1** and nictindole (L-8027).[1] In our screening of various pharmacological activities of pyrazolo[1,5-*a*]pyridines, it was observed that some of the compounds inhibited platelet aggregation. In the present study, we have investigated in detail the syntheses and antiaggregant activities of various kinds of pyrazolo[1,5-*a*]pyridine derivatives.



nictindole (L-8027)



I

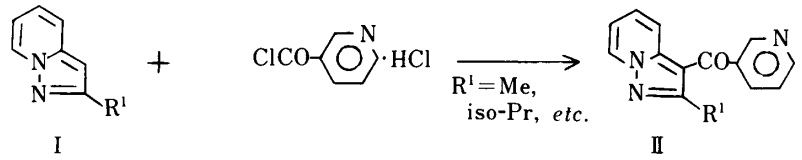
Chemistry

Three types of pyrazolo[1,5-*a*]pyridine derivatives were synthesized, as shown in Charts 1—3.

3-Nicotinoyl-2-substituted pyrazolo[1,5-*a*]pyridines (II) were synthesized by the reaction of 2-substituted pyrazolo[1,5-*a*]pyridine (I) with nicotinoyl chloride hydrochloride in nitrobenzene or 1,4-dioxane, at a temperature in the range of 100 to 160 °C (Chart 1).

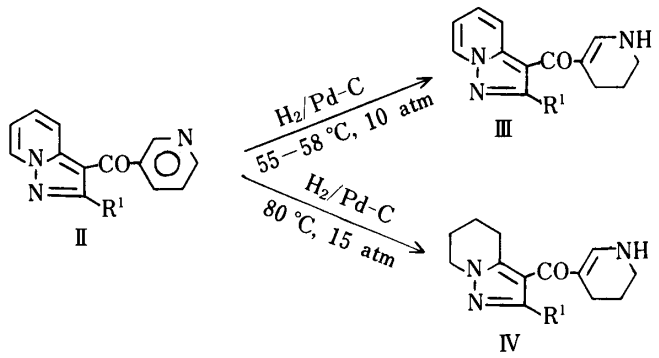
2-Substituted 3-(1,4,5,6-tetrahydronicotinoyl)pyrazolo[1,5-*a*]pyridines (III) and 2-substituted 3-(1,4,5,6-tetrahydronicotinoyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridines (IV) were obtained by the hydrogenation of II in ethanol, at the indicated hydrogen pressure and temperature, in the presence of palladium carbon (Chart 2). The structures of III and IV were assigned on the basis of the proton nuclear magnetic resonance (^{1}H-NMR) spin-decoupling technique. 2-Alkyl-3-(1-substituted 1,4,5,6-tetrahydronicotinoyl)pyrazolo[1,5-*a*]pyridines (V)

were prepared by the reaction of III with alkylating reagents in the presence of sodium hydride. Furthermore, compounds VI were prepared by the reaction of III with isocyanates in a similar way to that shown in Chart 3.



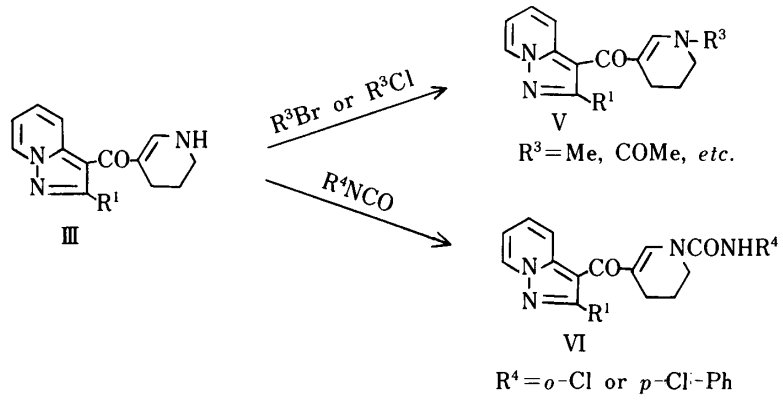
I II

Chart 1



II III IV

Chart 2



III V VI

R³=Me, COMe, *etc.*

R⁴=*o*-Cl or *p*-Cl-Ph

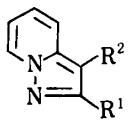
Chart 3

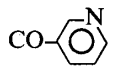
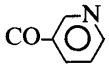
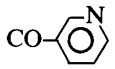
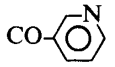
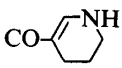
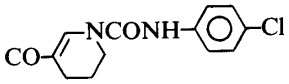
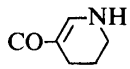
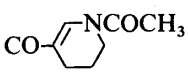
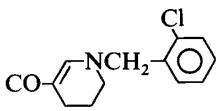
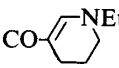
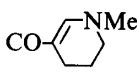
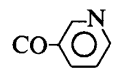
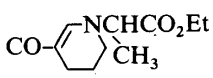
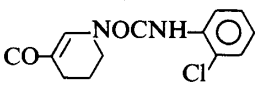
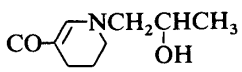
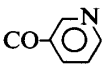
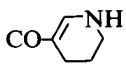
Inhibitory Activity on Arachidonic Acid-Induced Platelet Aggregation

All of the pyrazolo[1,5-*a*]pyridine derivatives in this study were tested for *in vitro* inhibitory activity on platelet aggregation induced by arachidonic acid in platelet-rich plasma from rabbit. The *in vitro* active compounds were also subjected to an *ex vivo* test.

The inhibitory activities of a series of pyrazolo[1,5-*a*]pyridine compounds are summarized in Tables I and II. Contrary to expectation, compound **1** exhibited little inhibitory activity (Table I). To examine the effect of substituents at the 2-position of **1**, the isopropyl group was replaced by methyl, methoxy, hydrogen and ethoxycarbonylmethoxy groups. These compounds showed no more activity than **1**, and even among the derivatives, only the methyl derivative (**4**) showed activity. This result suggested that the introduction of a methyl group at the 2-position of II increases the potency. Surprisingly, it was found that tetrahydronicotinoyl derivatives (III) showed markedly higher activity. Compound **5** was the most potent of these derivatives, being more potent than nictindole (L-8027) (Table I). This result confirmed that a methyl group was the most suitable substituent at the 2-position of III. In an attempt to find other compounds possessing higher activity, substitution of the

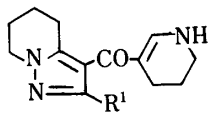
TABLE I. Physicochemical Properties and Inhibitory Activities on Platelet Aggregation



Compd. No.	R^1	R^2	mp (°C) (Yield (%))	Formula	Analysis (%) Calcd (Found)			Activity (ID_{100} μg/ml)
					C	H	N	
1	$CH(CH_3)_2$		90—92 (47)	$C_{16}H_{15}N_3O$	72.43 (72.47	5.70 5.59	15.84 15.77)	>10
2	$OCH(CH_3)CO_2Et$		— (69)	$C_{18}H_{17}N_3O_4$				>10
3	$OCH(CH_3)CO_2H$		121—123 (81)	$C_{16}H_{13}N_3O_4$	61.73 (61.84	4.21 4.08	13.50 13.39)	>10
4	CH_3		89—90 (61)	$C_{14}H_{11}N_3O$	70.87 (70.91	4.67 4.42	17.71 17.68)	3
5	CH_3		207—208 (63)	$C_{14}H_{15}N_3O$	69.69 (69.78	6.27 6.27	17.42 17.31)	0.03
6	CH_3		198—200 (73)	$C_{21}H_{19}ClN_4O_2$	63.88 (63.89	4.85 4.84	14.19 13.92)	1
7	$CH(CH_3)_2$		123—125 (87)	$C_{16}H_{19}N_3O$	71.34 (71.34	7.11 7.14	15.60 15.53)	1
8	CH_3		199—200 (51)	$C_{16}H_{17}N_3O_2$	67.82 (67.92	6.05 6.01	14.83 14.78)	>10
9	CH_3		134—135 (59)	$C_{21}H_{20}ClN_3O$	68.94 (68.90	5.51 5.34	11.49 11.58)	>10
10	CH_3		104—105 (72)	$C_{16}H_{19}N_3O$	71.34 (71.05	7.11 7.09	15.60 15.42)	>10
11	CH_3		139—140 (76)	$C_{15}H_{17}N_3O$	70.56 (70.56	6.71 6.68	16.46 16.37)	>10
12	H		218—219 (23)	$C_{13}H_9N_3O$	69.94 (69.95	4.06 3.78	18.83 18.85)	>10
13	CH_3		107—108 (59)	$C_{19}H_{23}N_3O_3$	66.84 (66.76	6.79 6.89	12.31 12.19)	0.3
14	CH_3		142—145 (47)	$C_{21}H_{19}ClN_4O_2$	63.98 (63.83	4.85 4.79	14.19 13.90)	>10
15	CH_3		169—170 (33)	$C_{17}H_{21}N_3O_2$	68.21 (68.00	7.07 7.09	14.04 13.95)	>10
16	OCH_3		201—203 (58)	$C_{14}H_{11}N_3O_2$	66.39 (66.33	4.38 4.22	16.59 16.38)	>10
17	OCH_3		263—265 (26)	$C_{14}H_{15}N_3O_2$	65.35 (65.05	5.88 5.86	16.33 16.00)	3
Aspirin								4
Nictindole								0.1

tetrahydronicotinoyl group at the *N*-position was carried out, but the activities were decreased, as shown in Table I (**6**, **8**—**11** and **13**—**15**). The α-ethoxycarbonyl derivative (**13**) was slightly less active than **5**, and other derivatives showed much weaker activity. We also

TABLE II. Physicochemical Properties and Inhibitory Activities on Platelet Aggregation



Compd. No.	R^1	mp (°C)	Yield (%)	Formula	Analysis (%) Calcd (Found) C	H	N	Activity (ID_{100} μg/ml)
18	CH_3	135—136	74	$C_{14}H_{19}N_3O$	68.54 (68.35	7.81 7.81	17.13 16.98)	0.2
19	$CH(CH_3)_2$	123—125	63	$C_{16}H_{23}N_3O$	70.29 (70.03	8.48 8.44	15.37 15.21)	1
20	OCH_3	196—198	58	$C_{14}H_{19}N_3O_2$	64.34 (64.24	7.33 7.51	16.08 15.75)	1

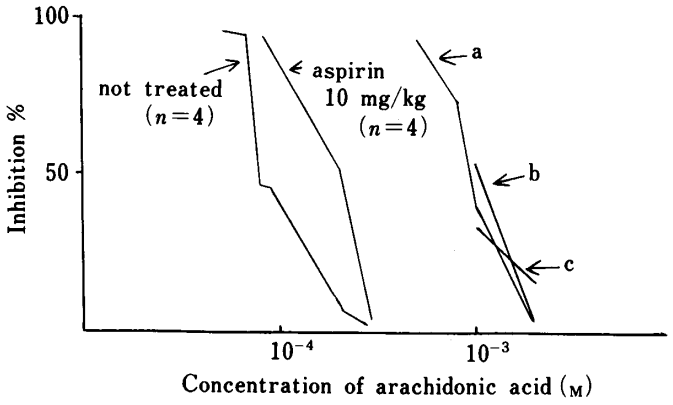


Fig. 1. Effect of **5** on the Arachidonic Acid-Induced Aggregation of Platelets from Rabbits Which Had Received the Drug Orally

a: compound **5**, 5 mg/kg ($n=3$).
b: compound **5**, 20 mg/kg ($n=3$).
c: compound **5**, 10 mg/kg ($n=4$).

synthesized tetrahydropyrazolo[1,5-*a*]pyridine derivatives (**18**—**20**), but these derivatives also showed decreased activity.

The most active compound **5** in this study was subjected to an *ex vivo* test to determine the absorbability and potency of the compound using platlet-rich plasma (PRP) from rabbit which had received the drug orally. The results (Fig. 1) indicate that **5** is several times more inhibitory than aspirin on arachidonic acid-induced platelet aggregation.

In conclusion, modification at the 2- and 3-position of **1** enhanced the inhibitory activity on platelet aggregation. In particular, it was found that the introduction of a methyl group at the 2-position and a 1,4,5,6-tetrahydronicotinoyl group at the 3-position gave a derivative that is more potent than nictindole or aspirin.

Experimental

Apparatus and Method——Melting points were measured with a Yanagimoto melting point apparatus and are uncorrected. NMR spectra were measured with a JNM-FX 90Q FT NMR spectrometer using tetramethylsilane as an internal standard. Column chromatography was carried out on silica gel (Wakogel C-200). Analyses within ±0.4% of theoretical values are indicated by the symbols of the elements alone. Platelet aggregation was measured in a Sienco DP-247E aggregometer.

Materials——Pyrazolo[1,5-*a*]pyridine and 2-isopropyl-, 2-methyl- and 2-hydroxypyrazolo[1,5-*a*]pyridine were prepared according to the reported methods.[2,3]

Preparation of 2-(α-Ethoxycarbonylethoxy)pyrazolo[1,5-*a*]pyridine——Ethyl α-bromopropionate (7.5 g) was added to a solution of 2-hydroxypyrazolo[1,5-*a*]pyridine (6 g) and anhydrous potassium carbonate (6.2 g) in acetone (160 ml) with stirring at reflux temperature. The resulting solution was refluxed for a further 16 h, and after removal

of the solvent *in vacuo*, the residue was dissolved in water (100 ml) and extracted with chloroform. The extract was dried over anhydrous Na_2SO_4, and after removal of the solvent, the residue was chromatographed over SiO_2 employing AcOEt–CH_2Cl_2 (1 : 4) as an eluent. The eluate was evaporated to dryness to afford the title compound as an oily product in a yield of 8 g (80%).

Preparation of 2-Methyl-3-nicotinoylpyrazolo[1,5-*a*]pyridine (4)——Nicotinoyl chloride hydrochloride (150 g) was added to a solution of 2-methylpyrazolo[1,5-*a*]pyridine (110 g) in 1,4-dioxane (600 ml) with stirring. The resulting solution was heated under reflux for 2 h, and the solvent was removed *in vacuo* (*ca.* 20 mmHg) by the use of a water-aspirator. The residue was dissolved in water (500 ml) and neutralized with potassium carbonate. The aqueous layer was extracted with benzene, the extract was dried over anhydrous Na_2SO_4, and after removal of the solvent, the residue was recrystallized from AcOEt–hexane to afford the title compound (110 g). Concentration of the mother liquor gave a second crop of crystals, which weighed 10 g; total yield 120 g (61%). Other nicotinoyl derivatives (**1**—**3**, **12**, **16**) were prepared in a similar manner. The yields and characteristics of the products are summarized in Table I.

Preparation of 2-Methyl-3-(1,4,5,6-tetrahydronicotinoyl)pyrazolo[1,5-*a*]pyridine (5) and 2-Methyl-3-(1,4,5,6-tetrahydronicotinoyl)-4,5,6,7-tetrahydropyrazolo[1,5-*a*]pyridine (18)——2-Methyl-3-nicotinoylpyrazolo[1,5-*a*]pyridine (10 g) was dissolved in absolute ethanol (150 ml) and 10% palladium carbon (2 g) was added to the solution. The mixture was hydrogenated in an autoclave under 10 atm of hydrogen and at a temperature of 55 to 58 °C for 3 h. The mixture was filtered and the filtrate was evaporated to dryness. The residue was recrystallized from ethyl acetate to afford the title compound **5** in a yield of 6.5 g (63%). ^{1}H-NMR ($CDCl_3$) δ: 1.82 (2H, m, nicotinoyl-5-H), 2.43 (2H, m, nicotinoyl-4-H), 2.44 (3H, s, CH_3), 3.23 (2H, m, nicotinoyl-6-H), 5.70 (1H, br, NH), 6.66 (1H, ddd, J=6.8, 6.6, 1.5 Hz, pyrazolopyridine-6-H), 7.07 (1H, s, nicotinoyl-2-H), 7.07 (1H, pyrazolopyridine-5-H), 7.49 (1H, dd, J=8.8, 1.5 Hz, pyrazolopyridine-4-H), 8.28 (1H, d, J=6.8 Hz, pyrazolopyridine-7-H). By following the same precedure as described for **5**, **18** was obtained by the hydrogenation (15 atm) of **4** at 80 °C. Other derivatives (**7**, **17**, **19**, **20**) were prepared in a similar manner (Tables I and II).

Preparation of 2-Methyl-3-(1-ethyl-1,4,5,6-tetrahydronicotinoyl)pyrazolo[1,5-*a*]pyridine (10)——Sodium hydride (55%, 0.3 g) was added in small portion to a solution of 2-methyl-3-(1,4,5,6-tetrahydronicotinoyl)pyrazolo[1,5-*a*]pyridine (1 g) in dimethylformamide (20 ml) with stirring. The stirring was continued at room temperature for 1 h, and then ethyl bromide was added. The reaction mixture was stirred at 60 °C for 2 h, then poured into ice-cold water and extracted with chloroform. The extract was dried over anhydrous Na_2SO_4, and after removal of the solvent, the reaction mixture was distilled to give the product, 18.7 g (84.2%). bp 136—137 °C (1.1 mm Hg). IR (neat) cm^{-1}: 1640 eluate was evaporated to dryness and the residue was recrystallized from benzene–hexane to afford the title compound in a yield of 0.8 g (72%). Other *N*-substituted derivatives (**8**, **9**, **11**, **13**) were prepared in a similar manner (Table I).

Preparation of 2-Methyl-3-[1-(*p*-chlorophenylcarbamoyl)-1,4,5,6-tetrahydronicotinoyl]pyrazolo[1,5-*a*]pyridine (6)——*p*-Chlorophenyl isocyanate (1 g) was added dropwise to a solution of 2-methyl-3-(1,4,5,6-tetrahydronicotinoyl)pyrazolo[1,5-*a*]pyridine (1 g) in dichloromethane (20 ml) under stirring. The mixture was stirred at room temperature for 2 h, and the solvent was removed *in vacuo* (*ca.* 20 mm Hg) by the use of a water-aspirator. The residue was recrystallized from ethyl acetate to afford the title compound in a yield of 1.2 g (73%). The *o*-chloro derivative (**14**) was prepared in the same way.

Preparation of 3-[1-(2-Hydroxypropyl)-1,4,5,6-tetrahydronicotinoyl]-2-methylpyrazolo[1,5-*a*]pyridine (15)——2-Methyl-3-(1,4,5,6-tetrahydronicotinoyl)pyrazolo[1,5-*a*]pyridine (1.35 g) and propylene oxide (0.98 g) were added to a solution of NaOH (0.02 g) in EtOH (20 ml) under stirring. The resulting solution was heated at 60 °C for 5 h, and after removal of the solvent *in vacuo*, the residue was dissolved in water. The aqueous layer was extracted with chloroform, the extract was dried over anhydrous Na_2SO_4, and after removal of the solvent, the residue was chromatographed over SiO_2 employing CH_2Cl_2–AcOEt–MeOH (8 : 1.5 : 0.5) as the eluent. The eluate was evaporated to dryness and the residue was recrystallized from AcOEt to afford the title compound in a yield of 0.55 g (33%).

Pharmacological Tests——Platelet aggregation was determined by the method using rabbit PRP.[4] The ID_{100} value is the concentration which gives maximum (100%) inhibition of platelet aggregation induced by arachidonic acid.

Ex Vivo Studies on Platelet Aggregation: The test compounds (**5** and aspirin) were orally administered to rabbits. One hour after the administration, blood was taken from the femoral artery. The methods for preparing PRP and determining platelet aggregation were the same as those used *in vitro*.[4] The results are given in Fig. 1.

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References

1) A. V. Prancan and J. Lefort, *Eur. J. Pharm.*, **60**, 287 (1979).
2) T. Irikura, Japan Kokai Tokkyo Koho 7654583 (1979).
3) H. Ochi, T. Miyasaka, K. Kanada and K. Arakawa, *Bull. Chem. Soc. Jpn.*, **49**, 1980 (1976).
4) G. V. R. Born and M. J. Cross, *J. Physiol.* (London), **168**, 178 (1963).