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Synthesis of *s*-Triazolo[1,5-*a*]pyrazine Derivatives and Cytokinin Activity[1]

Since the discovery of kinetin (6-furfurylaminopurine) in 1955, many compounds having cytokinin activity, *i.e.*, promoting cell division in tobacco pith callus in the presence of auxin, were reported.[2] Biologically active compounds have been found to be almost 6-substituted adenines. However, cytokinin activity of tetrazaindene derivatives other than those having a purine ring has not been studied.

In this communication, we wish to report the synthesis of *s*-triazolo[1,5-*a*]pyrazine derivatives and their cytokinin activity in the tobacco callus bioassay.

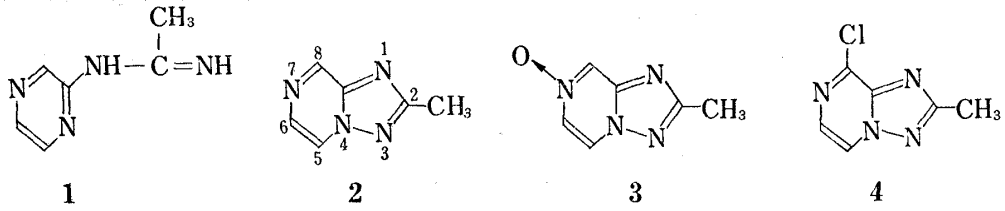
N-2-Pyrazylacetamidine[3] (**1**), mp 127°,[4] UV $\lambda_{max}^{95\%\ ethanol}$ mμ (ε): 258 (14000), 322 (8300), NMR ($CDCl_3$) τ: 1.27 (1H, d., J=1.5 cps, C_3-proton), 1.56 (1H, q., J=3 cps, J=1.5 cps, C_5 or C_6-proton), 1.66 (1H, d., J=3 cps, C_5 or C_6-proton), 7.72 (3H, s., $-CH_3$), was obtained by the reaction of 2-aminopyrazine with acetonitrile in the presence of aluminum chloride. This amidine was readily cyclised by lead tetraacetate in boiling acetic acid to 2-methyl-*s*-triazolo[1,5-*a*]pyrazine (**2**), mp 132°; UV $\lambda_{max}^{95\%\ ethanol}$ mμ (ε): 283 (4100), NMR ($CDCl_3$) τ: 0.63 (1H, d., J=2 cps, C_8-proton), 1.34 (1H, q., J=5 cps, J=2 cps, C_5 or C_6-proton), 1.71 (1H, d., J=5 cps, C_5 or C_6-proton), 7.27 (3H, s., $-CH_3$).

N-Oxydation of (**2**) with hydrogen peroxide in acetic acid gave 2-methyl-*s*-triazolo[1,5-*a*]pyrazine 7-oxide (**3**), mp 228 (decomp.), UV $\lambda_{max}^{95\%\ ethanol}$ mμ (ε): 247 (13500), 299 (9100), NMR (CH_3COOH) τ: 0.89 (1H, d., J=2 cps, C_8-proton), 1.16 (1H, d., J=6 cps, C_5 or C_6-proton), 1.80 (1H, q., J=6 cps, J=2 cps, C_5 or C_6-proton), which afforded (**2**) by catalytic reduction over Raney nickel.

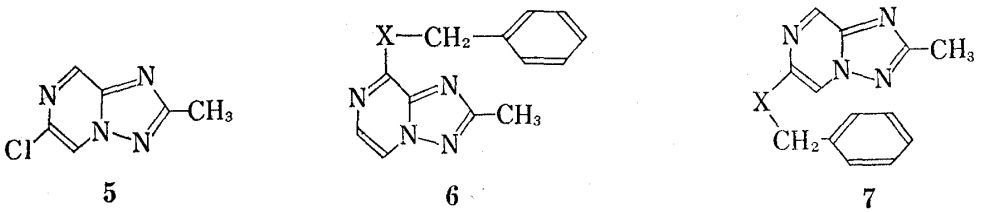
The reaction of (**3**) with phosphoryl chloride in boiling chloroform gave two isomeric chloro derivatives, 2-methyl-8-chloro-*s*-triazolo[1,5-*a*]pyrazine (**4**), mp 111.5—112°, UV $\lambda_{max}^{95\%\ ethanol}$ mμ (ε): 292 (5000), NMR ($CDCl_3$) τ: 1.57 (1H, d., J=4.5 cps, C_5 or C_6-proton), 2.08 (1H, d., J=4.5 cps, C_5 or C_6-proton), 7.30 (3H, s., $-CH_3$), and 2-methyl-6-chloro-*s*-triazolo[1,5-*a*]-pyrazine (**5**), mp 112°, UV $\lambda_{max}^{95\%\ ethanol}$ mμ (ε): 294 (5200), NMR ($CDCl_3$) τ: 0.84 (1H, s., C_5 or C_8-proton), 1.76 (1H, s., C_5 or C_8-proton), 7.22 (3H, s., $-CH_3$).

Condensation of (**4**) with benzylamine in butanol, benzyl alcohol, or benzylthiol in methanol provided 2-methyl-8-benzylamino-*s*-triazolo[1,5-*a*]pyrazine (**6a**), mp 136—137°, UV $\lambda_{max}^{95\%\ ethanol}$ mμ (ε): 260 (11200), 266 (10700), 303 (7500), NMR ($CDCl_3$) τ: 2.24 (1H, d., J=4.5 cps, C_5 or C_6-proton), 2.50 (1H, d., J=4.5 cps, C_5 or C_6-proton), 2.68 (5H, s., benzene ring protons), 3.70 (1H, broad, -NH-), 5.23 (2H, d., J=6 cps, $-CH_2-$), 7.43 (3H, s., $-CH_3$), 2-methyl-8-benzyloxy-*s*-triazolo[1,5-*a*]pyrazine (**6b**), mp 93—94°, UV $\lambda_{max}^{95\%\ ethanol}$ mμ (ε): 243 (4600), 283 (5700), NMR ($CDCl_3$) τ: 1.99 (1H, d., J=4.5 cps, C_5 or C_6-proton), 2.50 (1H, d., J=4.5 cps, C_5 or C_6-proton), 2.66 (5H, m., benzene ring protons), 4.42 (2H, s., $-CH_2-$), 7.41 (3H, s., $-CH_3$), and 2-methyl-8-benzylthio-*s*-triazolo[1,5-*a*]pyrazine (**6c**), mp 76—77°, UV $\lambda_{max}^{95\%\ ethanol}$ mμ (ε): 250 (8800), 310 (10900), NMR ($CDCl_3$) τ: 1.88 (1H, d., J=4.5 cps, C_5 or C_6-proton), 2.12 (1H, d., J=4.5 cps, C_5 or C_6-proton), 2.70 (5H, m., benzene ring protons), 5.44 (2H, s., $-CH_2-$), 7.38 (3H, s., $-CH_3$).

1) A part of this work was presented at the 86th Annual Meeting of the Pharmaceutical Society of Japan, Sendai, October 1966 (Abstr. Papers, p. 94).
2) C.O. Miller, *Ann. Rev. Plant Physiol.*, **12**, 395 (1961); E.M. Shanz, *ibid.*, **17**, 409 (1966). D.S. Letham, *ibid.*, **18**, 349 (1967); F. Skoog and N.J. Leonard, *Phytochemistry*, **6**, 1169 (1967).
3) Satisfactory elemental analyses were obtained for all the new compounds reported here.
4) All melting points were measured on Yanagimoto micro-melting point measuring apparatus and are uncorrected.



1 2 3 4



5 6 7

6a : X=NH
6b : X=O
6c : X=S

7a : X=NH
7b : X=O
7c : X=S

Chart 1

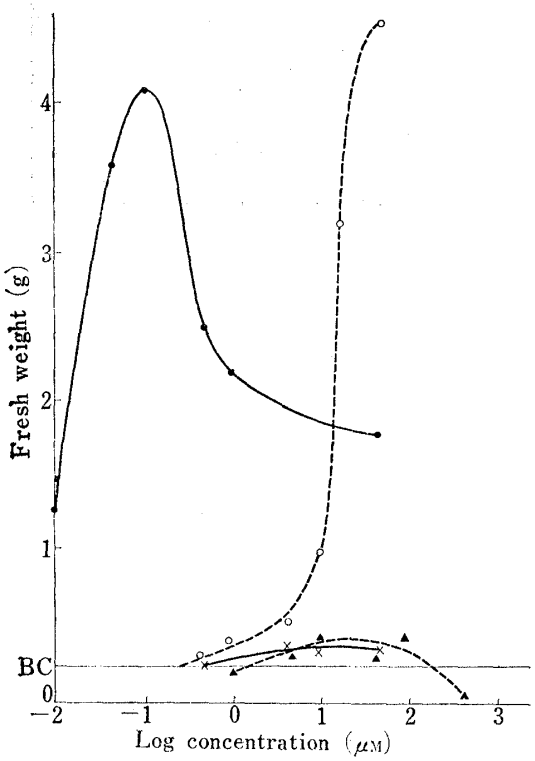


Fig. 1. Effect of Concentration of **6a**, **7a**, and **7c** on Yield of Tobacco Callus

Ordinate : mean value of twelve calluses
BC : basal medium control
(Low solubility of compound **6a** and **7c** prevented its being tested in a concentration higher than 41 μM.)
—●— Kinetin control ···▲··· **7a**
···○··· **6a** —×— **7c**

In a similar manner, 2-methyl-6-benzylamino-*s*-triazolo[1,5-*a*]pyrazine (**7a**), mp 131°, UV $\lambda_{max}^{95\%\ ethanol}$ mμ (ε): 268 (5600), 329 (14400), NMR ($CDCl_3$) τ: 1.48 (1H, s., C_5 or C_8-proton), 2.59 (1H, s., C_5 or C_8-proton), 2.66 (5H, s., benzene ring protons), 4.00 (1H, broad, -NH-), 5.40 (2H, d., J=6 cps, $-CH_2-$), 7.38 (3H, s., $-CH_3$), 2-methyl-6-benzyloxy-*s*-triazolo[1,5-*a*]pyrazine (**7b**), mp 182°, UV $\lambda_{max}^{95\%\ ethanol}$ mμ (ε): 257 (3200), 300 (9200), NMR ($CDCl_3$) τ: 1.23 (1H, s., C_5 or C_8-proton), 2.35 (1H, s., C_5 or C_8-proton), 2.58 (5H, s., benzene ring protons), 4.50 (2H, s., $-CH_2-$), 7.31 (3H, s., $-CH_3$), and 2-methyl-6-benzylthio-*s*-triazolo[1,5-*a*]pyrazine (**7c**), mp 97—98°, UV $\lambda_{max}^{95\%\ ethanol}$ mμ (ε) 250 (16800), 335 (2100), NMR ($CDCl_3$) τ: 0.91 (1H, d., J=1.5 cps, C_5 or C_8-proton), 1.69 (1H, d., J=1.5 cps, C_5 or C_8-proton), 2.74 (5H, s., benzene ring protons), 5.63 (2H, s., $-CH_2-$), 7.38 (3H, s., $-CH_3$), were prepared from the compound (**5**).

The cytokinin activity of these compounds was assayed by the method similar to that reported by Murashige and Skoog[5] with the exception of the following points: Tobacco (*Nicotiana tabacum* var. *Bright Yellow*)[6] plant cultivated in a growth chamber (30° with nearly 10000 lux of fluorescent light mixed with incandecent light) was always employed.

5) T. Murashige and F. Skoog, *Physiol. Plantarum*, **15**, 473 (1962).
6) This test was also carried out with *Nicotiana tabacum* var. Wisconsin No. 38 and the same result was obtained.

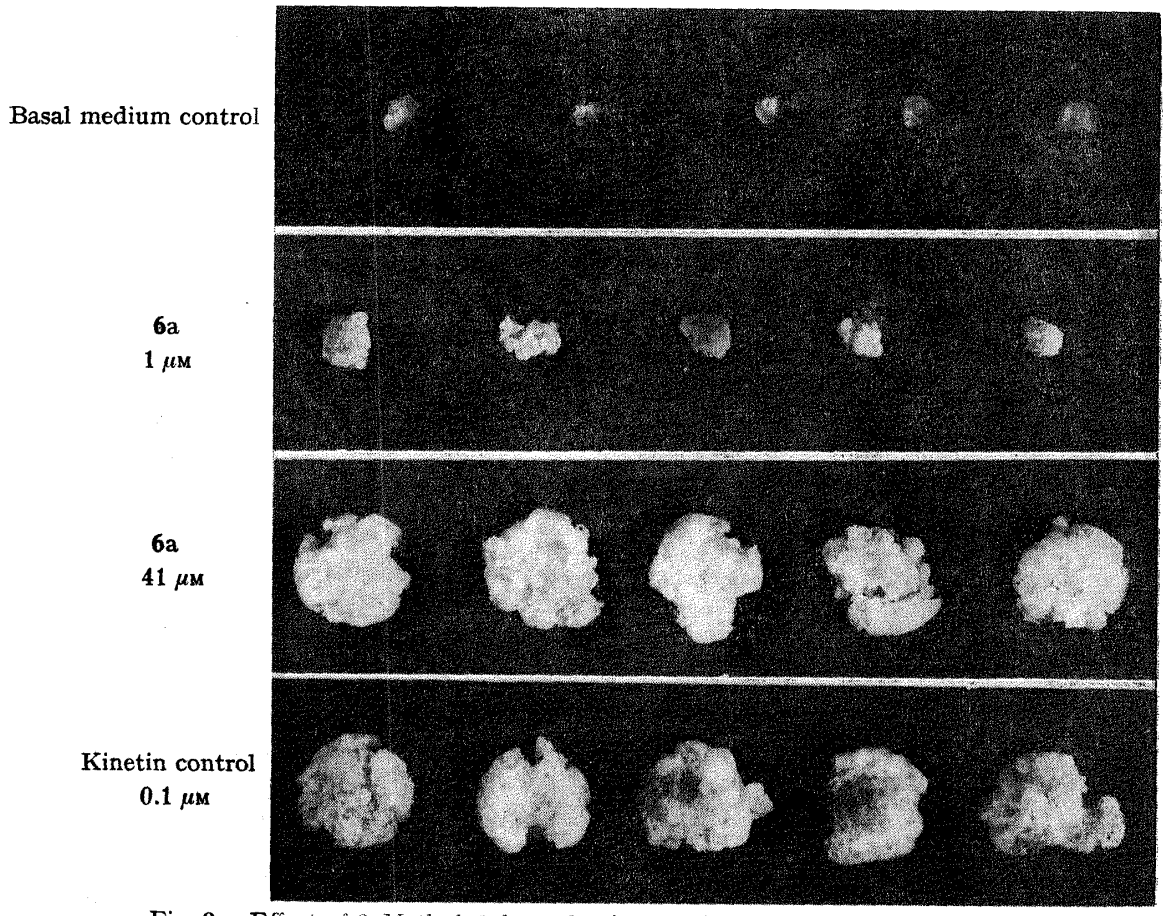


Fig. 2. Effect of 2-Methyl-8-benzylamino-*s*-triazolo[1,5-*a*]pyrazine (**6a**) and Kinetin on Growth of Tobacco Callus

The test was carried out using one batch of 12 test tubes (3 cm in diameter and 20 cm in length) each of which contained 20 ml of culture medium dissolving various concentartions of these compounds. Each tube was planted with 50—60 mg of fresh weight of the 2nd generation of pith callus and the tubes were kept at 26° under weak diffused light for 30 days and fresh weight of the callus was measured.

As shown in Fig. 1 and 2, (**6a**) has a marked activity, while (**7a**) and (**7c**) are weakly active, while (**6b**), (**6c**) and (**7b**) are almost inactive.

Further studies on chemical reactivity of this *s*-triazolo[1,5-*a*]pyrazine ring and synthesis of other tetrazaindene derivatives are now in progress.

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