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Chemical Studies on 4-Methylthio-3-Butenyl Isothiocyanate from Roots of Japanese Radish(*Raphanus sativus* L.) in connection with Raphanusanins, Phototropism-Regulating Substances of Radish Hypocotyls

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Key words: 4-methylthio-3-butenyl isothiocyanate; raphanusanins; growth inhibitor; phototropism; radish hypocotyls

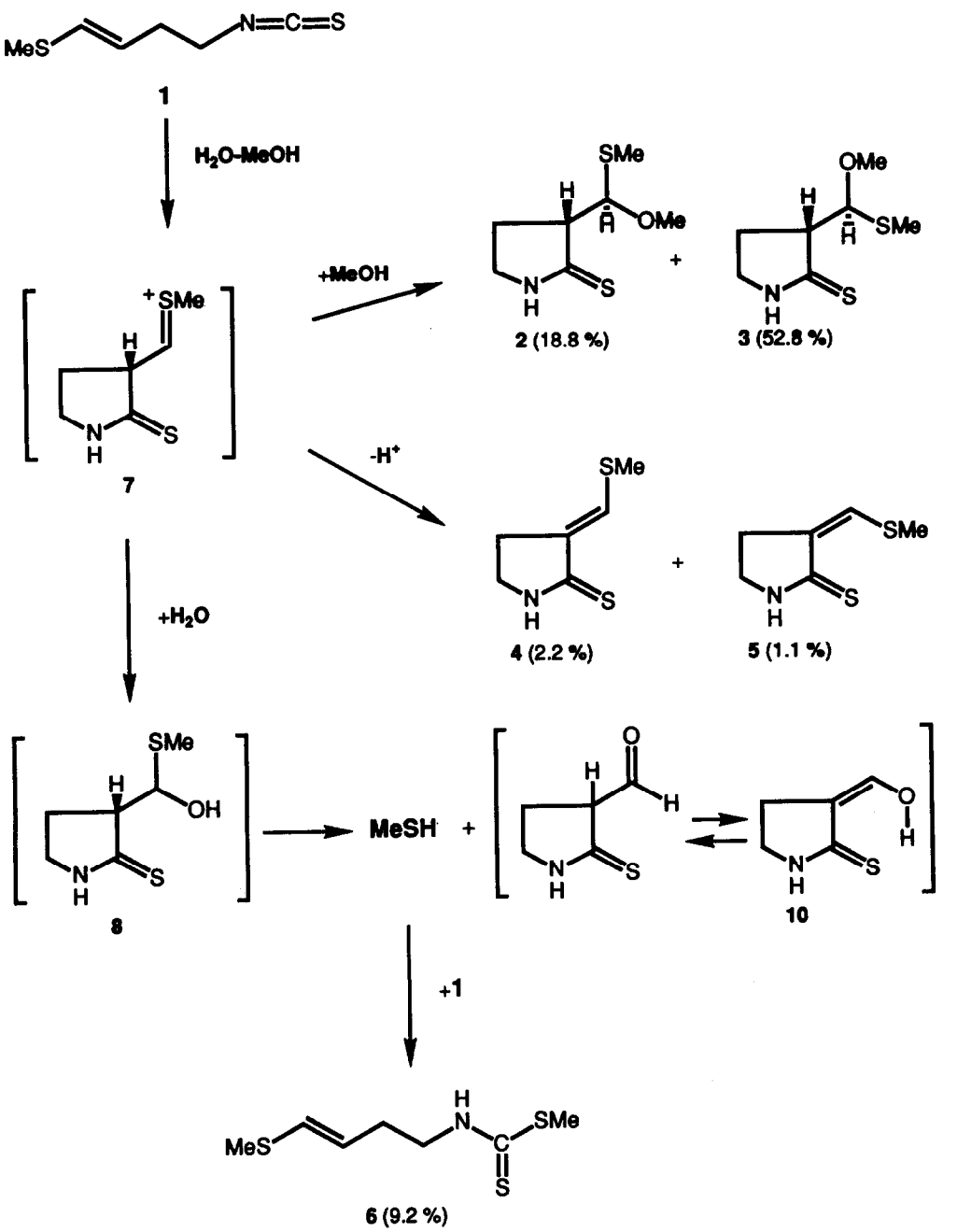
Summary: 4-Methylthio-3-butenyl isothiocyanate as the direct precursor of raphanusanins has been isolated from the commercially available radish (*Raphanus sativus* L.), and some chemical studies on 4-methylthio-3-butenyl isothiocyanate (MTB-ITC) have been carried out, where it has been spontaneously converted into raphanusanins, phototropism-regulating substances of radish hypocotyls, in MeOH-H_2O as well as in H_2O solution.

In connection with raphanusanins, phototropism-regulating substances of radish (*Raphanus sativus* var. *hortensis* f. *gigsntissimus* Makino) hypocotyls, some novel metabolites have been isolated from light-grown radish seedling.[1] In this communication we wish to report the isolation and structural identification of the precursor of raphanusanins, 4-methylthio-3-butenyl isothiocyanate (MTB-ITC), which was spontaneously converted into raphanusanins *in vitro*.

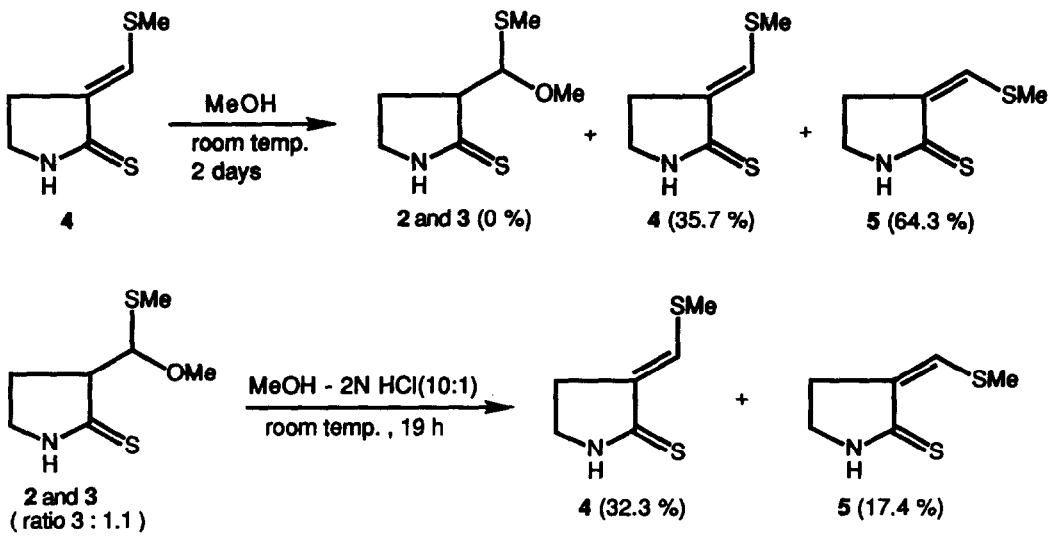
The commercially available fresh roots of radish (*Raphanus sativus* L.) (4 Kg) were grated by a food processor at room temperature, and then was directly pressed out and shaken with hexane. The hexane extract (1,050 mg) was directly separated by preparative TLC (Kieselgel PF_{254}) using hexane-AcOEt (4:1) to afford the direct precursor of raphanusanins, MTB-ITC (**1**) in 0.018 % yield, wherein raphanusanins A and B (**2** and **3**) previously isolated in a racemic form [1] were not detected. The spectral data of the precursor (**1**) of raphanusanins are shown below.

The precursor (**1**) of raphanusanins as a colorless oil: $C_6H_9NS_2$[m/z 159.0180(M^+)]; IR(film) 2170 and 2085 cm^{-1}; $\delta_H(CDCl_3)$ 2.27 (3H, s), 2.50 (2H, dt, J=7.1, 6.6Hz), 3.53 (2H, t, J=6.6Hz), 5.35 (1H, dt, J=15.0, 7.1Hz), and 6.20 (1H, d, J=15.0Hz); $\delta_C(CDCl_3)$ 14.71 (q), 33.83 (t), 45.11 (t), 120.07 (d), 129.13 (d), and 131.37 (s).

As judged from these spectral data, this compound (**1**) is proved to be 4-methylthio-3-butenyl isothiocyanate which has already been isolated as a pungent principle in root of Japanese radish (*Raphanus sativus* L.).[2] However, MTB-ITC (**1**) has not yet been known as a precursor of growth inhibitor and any chemical conversion of **1** to raphanusanins has not been carried out.


MeS
N=C=S
1
H2O-MeOH
+MeOH
SMe
OMe
2 (18.8 %)
3 (52.8 %)
7
-H+
4 (2.2 %)
5 (1.1 %)
+H2O
OH
8
MeSH
10
+1
6 (9.2 %)

Scheme 1



Scheme 2

On the basis of biogenetic consideration, biomimetic reaction of MTB-ITC has been carried out, as follows. Although MTB-ITC is stable in acetone, benzene or hexane solution at room temperature for one week, it was easily converted into raphanusanin A (**2**) (18.8 %), raphanusanin B (**3**) (52.8 %), 3-*trans*-(methylthio)-methylene-2-pyrrolidinethione (**4**) (2.2 %),[3] 3-*cis*-(methylthio)-methylene-2-pyrrolidinethione (**5**) (1.1 %)[4] and compound **6** (9.2 %)[5] in MeOH-H_2O (1:1) at room temperature for 24 h (see Scheme 1).[6] When sonicated with H_2O at room temperature for 4 h, MTB-ITC (**1**) was converted into **4** (1.8 %), **5** (1.3 %), **6** (7.9 %) and many structurally unidentified compounds (probably, some compounds have a five-membered thiolactam moiety). In the presence of a small amount of H_2SO_4,[7] the intramolecular cyclization reaction was accelerated to afford compounds **4** and **5** in higher yields, while the yield of the compound **6** was decreased. Furthermore, **4** was isomerized to the corresponding cis-isomer (**5**) (64.3 %) and not converted into **2** and/or **3** in MeOH at room temperature for 2 days, and a mixture of raphanusanins A and B (ratio : 3 / 1.1) was treated with 2N HCl-MeOH (10:1) (room temp., 19 h) to afford two olefinic compounds (**4** and **5**) in 32.3 and 17.4 % yields, respectively (see Scheme 2).

In the light of these chemical evidences and a number of synthetic analogues[8,9] with a five or six-membered thiolactam moiety which have been proved to inhibit the hypocotyl growth of etiolated lettuce seedlings, from a view point of phototropism, 4-methylthio-3-butenyl glucosinolate (**11**)[7] could be formed at the light exposure side, and then hydrolyzed with endogeneous myrosinase to afford MTB-ITC (**1**) which is spontaneously converted into some five-membered thiolactams (**4**, **5** and others) and **6** *in vivo*, wherein both **1** and **11** have no growth inhibitory activity against lettuce hypocotyls, while the resulting thiolactams interestingly inhibit the hypocotyl growth of radish. Further studies on this point are in progress.

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3. **4** as pale yellow amorphous powder: $C_6H_9NS_2$[m/z 159.0148(M+)]; IR(film)3150, 1600, and 1530cm^{-1}; $\delta_H(CDCl_3)$ 8.14(1H,br.s), 7.58(1H, t, J=2.7Hz), 3.68(2H, dt, J=1.0, 7.1Hz), 2.79(2H, dt, J=2.7, 7.1Hz), and 2.52(3H, s).
4. **5** as pale yellow amorphous powder: $C_6H_9NS_2$[m/z 159.0175(M+)]; IR(film)3150, 1600, and 1530cm^{-1}; $\delta_H(CDCl_3)$ 7.85(1H, br.s), 6.75(1H, t, J=1.7Hz), 3.62(2H, dt, J=1.0, 7.3Hz), 3.00(2H,dt, J=1.7, 7.3Hz), and 2.40(3H, s).
5. **6** as a colorless oil: $C_7H_{13}NS_3$[m/z 207.0183(M+)]; IR(film)3250, 1600, and 1500cm^{-1}; $\delta_H(CDCl_3)$ 6.94(1H, br.s, NH), 6.15(1H, d, J=14.7Hz), 5.34(1H, dt, J=6.8, 14.7Hz), 3.79(2H, dt, J=6.8, 6.8Hz), 2.63(3H, s), 2.48(2H, dt, J=6.8, 6.8Hz), and 2.26(3H,3); $\delta_C(CDCl_3)$ 199.06(s), 127.69(d), 121.65(d), 46.45(t),31.83(t), 18.09(q), and 14.75(q). This compound (**6**) was isolated from the radish roots in 0.00011 % yield, and inhibited the hypocotyl growth of etiolated lettuce seedlings.
6. Any amount of **10** has not been detected under this condition, but small amount of **10** has been obtained by Y. Uda et al. (*Agric. Biol. Chem.*, **1990**, *54*, 613).
7. When the cells of tissues are crushed, 4-methylthio-3-butenyl isothiocyanate (**1**) is released from 4-methylthio-3-butenyl glucosinolate (**11**) by the action of enzyme myrosinase(thioglucosidase) along with *inorganic sulphate* and glucose. In fact, on treatment of radish with boiling water for a few minutes, compound **11** was mainly obtained instead of **1**.

11

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