tion of V was executed, according to Greenwood, *et al.*,[5] and followed by purification through SP-Sephadex C25 column.

The radioimmunoassay procedure is as follows. 100 μl of urine or Tris-HCl buffered saline(0.05 M, pH 7.4), containing various amounts of unlabelled methamphetamine, was submitted to incubation at 37° for 1 hr with 300 μl of the antiserum diluted 900 times by the same buffered saline as above, which contains a fixed amount of the ^{125}I-labelled compound (*ca.* 50000 cpm). An equal volume of satd. $(NH_4)_2SO_4$ solution was added after the incubation to all reaction mixtures. After centrifugation at 3000 rpm for 15 min at 5°, the activity of the supernatant containing the unbound labelled compound was conuted as usual. The sensitivity of the present immunoassay proves to be 8.0 ng/100 μl. To examine the specificity of the antiserum several kinds of substituted phenethylamines were tested for their abilities of inhibition of the binding of the labelled compound with the antibody. The results are exhibited in Fig. 2 together with the standard curve of I.

An illegal and non-medical consumption of more than 10 mg of I have been prevailing in a certain community indulged with I by its abuse. It was reported that the most abundunt excrement into urine in man is the unchanged I, and the rate of the excretion is calculated approximately 20% per 24 hr.[6] Therefore, the concentration of I in the urine collected within 24 hr (totally 1—2 liter) seems to be estimated about 1.0—2.0 μg/ml. In conclusion, since this new method for determination of I has revealed the sensitivity of 8.0 ng/ 100 μl, it may be expected for application in the field of forensic sciences.

A further study on multiplication of sensitivity of the method is now under progress.

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5) F. Greenwood, W. Hunter, and J. Glover, *Biochem. J.*, **89**, 114 (1963).
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A New Synthesis of Dihydromancunine

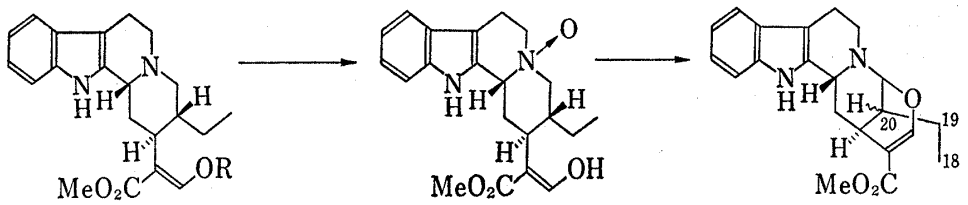
Dihydromancunine, a model for a proposed indole alkaloid biosynthetic intermediate, mancunine, was synthesized using a modified Polonovski reaction of desmethylhirsutine N-oxide, which was derived from the indole alkaloid hirsutine.

Keywords——indole alkaloid; biosynthetic intermediate; biomimetic synthesis; Polonovski reaction; N-oxide

In 1974, Brown and co-workers[1] reported the synthesis of dihydromancunine (**4**), a model for a proposed indole alkaloid biosynthetic intermediate, mancunine, from dihydro-

1) R.T. Brown, C.L. Chapple, and A.A. Charalambids, *Chem. Commun.*, **1974**, 756.

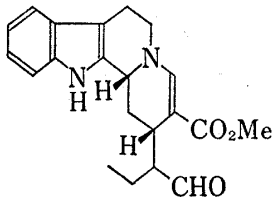
vincoside, and its conversion into the Corynanthé indole alkaloid derivative dihydrositsirikine. They also synthesised **4** by a one-pot biomimetic method from dihydrosecologanin and tryptamine using β-glucosidase.[2)]



1 : R=CH_3
2 : R=H

3

4 : C_{20}-Hα
5 : C_{20}-Hβ



6

Chart 1

We wish to report a new biomimetic synthesis of 4 using a modified Polonovski reaction of desmethylhirsutine N-oxide (**3**). Desmethylhirsutine (**2**)[3)] was oxidized in about 70% yield to the N-oxide (**3**) [mp 152—153°, ultraviolet spectrum (UV): indolic chromophore in neutral solution, λ_{max}^{basic} 223, 274 nm (with increased intensity, enolic chromophore of β-hydroxy acrylic ester)], which could be reduced to the starting compound **2** with $NaHSO_3$ by refluxing in aq MeOH solution under N_2. The modified Polonovski reaction of the N-oxide compound (**3**) was accomplished in the following manner. Thus, **3** was added to excess trifluoroacetic anhydride in CH_2Cl_2 at O°. After about 3 hr, removal of the solvent was made under reduced pressure and the residue separated by column chromatography on silica gel using a benzene–chloroform system. An early fraction gave a crystalline compound A, mp 180—181° (dec.) from ether, in 33% yield. UV spectrum of A showed the indolic chromophore and no shift on addition of aq NaOH. The mass spectrum of A exhibited M^+ at *m/e* 352 which indicated loss of 2 mass units from **2**. The presence of a β-alkoxy acrylic ester group in A was revealed by the nuclear magnetic resonance (NMR) spectrum [$C\underline{H}_3OOC$–$\dot{C}$=$C\underline{H}$–O–$\dot{C}\underline{H}$–N, δ 3.75(3H, singlet), 7.82(1H, s.) and 4.90(1H, s.)] and infrared (IR) spectrum [O=$\dot{C}$–$\dot{C}$=$\dot{C}$–O–, 1690, 1620 cm^{-1}]. The optical rotation of A showed a very high value, $[\alpha]_D^{23}$: +322 ($CHCl_3$). As these spectral data were similar to those of dihydromancunine (**4**) ,direct comparison of compound A with an authentic specimen[4)] of **4** was made. They were found to be identical in all respects (IR, NMR, mass spectra and mixture melting point). Compound A was shown to be in equilibrium with a trace of 20β-dihydromancunine (**5**) by thin layer chromatography (TLC). After separation of **5** by preparative TLC, this re-equilibrated to give mainly dihydromancunine (**4**) as shown by TLC, and a further rearranged derivative 19,20-dihydroisovallesiachotamine (**6**)[1)] [amorphous, λ_{max}^{EtOH} 223, 292 nm, mass *m/e* (%): 352 (M^+, 13), 324 (M^+–CO, 12), 281(100)], formed as a by-product of this treatment.

2) R.T. Brown, C.L. Chapple, R. Platt, and S.K. Sleigh, *Tetrahedron Lett.*, **1976**, 1829.
3) S. Sakai and N. Shinma, *Chem. Pharm. Bull.* (Tokyo), **22**, 3013 (1974).
4) We thank Dr. R.T. Brown, Department of Chemistry The University of Manchester, for generous gift of dihydromancunine and also for a private communication about reference 2 prior to publication.

Attempts to synthesise the hypothetical biogenetic intermediate mancunine (**5**, 18,19-double bond) from hirsuteine (**1**, 18,19-double bond) by the same route were unsuccesful.

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Application of ^{13}C NMR Spectroscopy to Chemistry of Natural Glycosides: Rebaudioside-C, a New Sweet Diterpene Glycoside of *Stevia rebaudiana*

From leaves of *Stevia rebaudiana* BERTONI (Compositae), there were isolated three new sweet glycosides, named rebaudioside-C, -D, and -E. Application of ^{13}C NMR spectroscopy as well as chemical evidences led to assign the structure V in Chart 1 to rebaudioside-C.

Keywords——^{13}C NMR of oligoglycosides; PRFT method; Kaurene type diterpenes; rebaudiosides-C, -D, -E; natural sweetener; *Stevia rebaudiana* BERTONI; Compositae

Stevia rebaudiana BERTONI (Compositae), a wild herb of Paraguay is known to contain the sweet glucoside, stevioside(I)[1] and has attracted much attention as a new source of the natural sweetener. Recently, the present authors reported isolation and structural determination of additional sweet glucosides, named rebaudiosides-A(II) and -B(III) from this plant.[2]

The glycoside fraction[2] of the methanolic extract of leaves of this plant was recrystallized from methanol to give I and the mother liquor was subjected to repeated column chromatography on silica gel (solvent $CHCl_3$: MeOH: H_2O(30: 10: 1 homogeneous) and AcOEt: MeOH(10: 1)), affording now three new sweet glycosides, named rebaudiosides-C (yield 0.4%), –D(yield 0.03%) and –E(yield 0.03%) along with I, II, III and steviolbioside-(IV).[2]

Rebaudioside-C(V), colorless needles, mp 215—217°, $[\alpha]_D^{25}$ −29.9° (MeOH) which was crystallized by slow concentration of its methanolic solution, showed its spot[3] between those of I and II on thin-layer chromatogram on silica gel(solvent $CHCl_3$: MeOH: H_2O(15: 6: 1 homogeneous)).

On the basis of our recent study on ^{13}C nuclear magnetic resonance(CMR) of *Stevia* diterpene glycosides,[4] the spectrum[5] of V revealed that V must be a glycoside of steviol(VI), both the 19-COOH and the 13-*tert*-OH of which must be combined with sugar moieties. On hydrolysis with crude hesperidinase,[6] V afforded glucose, rhamnose and VI, while alkaline

1) E. Mosettig, U. Beglinger, F. Dolder, H. Lichiti, P. Quitt, and J.A. Waters, *J. Am. Chem. Soc.*, **85**, 2305 (1963) and the references cited therein.
2) H. Kohda, R. Kasai, K. Yamasaki, K. Murakami, and O. Tanaka, *Phytochemistry*, **15**, 981 (1976).
3) Visualized as a yellow spot on heating after spraying 10% H_2SO_4.
4) K. Yamasaki, H. Kohda, T. Kobayashi, R. Kasai, and O. Tanaka, *Tetrahedron Letters*, **1976**, 1005.
5) All spectra were taken in C_5D_5N; δ ppm from TMS; at 25°.
6) H. Kohda and O. Tanaka, *Yakugaku Zasshi*, **95**, 246 (1975).