IIIb: prisms, mp 142—143° (dec.). Yield, 65%. IR ν_{max}^{KBr} cm^{-1}: 3284 (NH), 1674, 1528 (CONH), 1612, 1400 (COO$^-$). *Anal.* Calcd. for $C_{19}H_{21}N_3O_4$: C, 64.21; H, 5.96; N, 11.83. Found: C, 64.37; H, 6.05; N, 11.83.

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Studies on Constituents of Medicinal Plants. XIX.[1] Constituents of *Schizandra nigra* Max. (3)

Masako Takani, Machiko Nakano, and Kōtaro Takahashi

Faculty of Pharmaceutical Sciences, Kanazawa University[2]

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(+)-Catechin-7β-D-glucopyranoside was isolated from the wooden part of *Schizandra nigra* Max.

Keywords——(+)-catechin-7β-D-glucopyranoside; *Schizandra nigra* Max.; NMR; MS; magnoliaceae

The authors have previously isolated schizandronic acid,[3] schizandrolic acid,[4] schizandronol[4] and oplodiol[4] from the methanol-soluble fraction of the wooden part of *Schizandra nigra* Max. and elucidated the structures of the former three. This paper concerns with the isolation and the structural elucidation of a new (+)-catechin glucoside. As the (+)-catechin type glycoside, (+)-catechin-7-L-arabinoside,[5] (+)-catechin 5β-D-xylopyranoside,[6] (+)-catechin-7 β-D-xylopyranoside[7] and (+)-catechin-7 α-L-rhamnopyranoside[8] have been reported.

The methanol-soluble fraction afforded a compound (I), $C_{21}H_{24}O_{11}\cdot 1\frac{1}{2}H_2O$, colorless needles of mp 215—216°, $[\alpha]_D^{32}=-33.4$ (c=1.0, MeOH). Compound I gave green coloration with $FeCl_3$ and shows the ultra-violet (UV) absorption maximum at 281.5 nm (log ε 3.56) and the infra-red (IR) absorption bands (cm^{-1}) at 3500—3000 (OH), 1620, 1600 (benzene ring), 1170—1030 (-C-O-). Compound I afforded octaacetyl derivative $C_{37}H_{40}O_{19}$ (II), colorless needles of mp 130° on acetylation with acetic anhydride and pyridine, and trimethyl derivative $C_{24}H_{30}O_{11}\cdot H_2O$ (III), colorless needles of mp 171—174° on methylation with diazomethane, but I did not afford tetramethyl derivative on methylation with diazomethane.

On enzymatic hydrolysis with β-glycosidase (emulsin), III afforded D-glucose and a compound $C_{18}H_{20}O_6$ (IV), colorless plates of mp 261—264° and IV afforded a compound $C_{19}H_{22}O_6$ (V), colorless needles of mp 145—147° by methylation with diazomethane. The compounds

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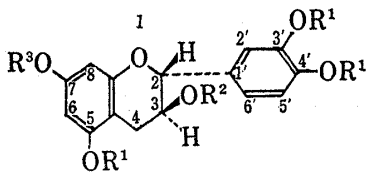
IV and V were proved to be identical with 5,3′,4′-trimethyl-(+)-catechin[5] and 5,7,3′,4′-tetramethyl-(+)-catechin[9] by the mixed fusion, IR and thin layer chromatogram (TLC), respectively. These chemical and spectral evidences indicate that I is (+)-catechin-7β-D-glucopyranoside as shown in chart 1. The nuclear magnetic resonance (NMR) spectra of II, III, IV and V were interpreted as described in the experimental part.



I : $R^1=R^2=H$, $R^3=\beta$-D-glucopyranosyl,
II : $R^1=R^2=OAc$, $R^3=$tetra-O-acetyl-β-D-glucopyranosyl,
III : $R^1=Me$, $R^2=H$, $R^3=\beta$-D-glucopyranosyl,
IV : $R^1=Me$, $R^2=R^3=H$,
V : $R^1=R^3=Me$, $R^2=H$,

Chart 1

Experimental

The following instruments were used for the physical data. Melting point: Yanagimoto Micro-Melting apparatus (a hot-plate type); UV spectra: Hitachi 323 recording spectrometer in ethanol; IR spectra (cm^{-1}): Nippon Bunko IR-G spectrometer in KBr; NMR spectra (δ value, ppm): JNM-PS-100 high resolution instrument at 100 MHz with $(CH_3)_4Si$ as an internal reference; mass spectra (MS): JMS-OISG mass spectrometer; optical rotation: Nippon Bunko automatic polarimeter DIP-SL at 589 nm. TLC was obtained on a glass plate coated with silica gel G (Merck). Abbreviation: s, singlet; d, doublet; q, quartet; m, multiplet.

Isolation of I——Dried cut wooden part (5 kg) of *Schizandra nigra* was extracted with methanol and the extract was chromatographed on a column of silica gel with *n*-hexane, benzene, $CHCl_3$, ethyl acetate and acetone, successively. The acetone-soluble fraction was chromatographed on a column of silica gel with $CHCl_3$–MeOH (3: 1). The fraction of *Rf* 0.23 (TLC, $CHCl_3$: MeOH=3: 1) was chromatographed on a column of celite-active charcoal (2: 1) with $CHCl_3$–MeOH (3: 1) to afford crystalline powder (yield 4.7%), which was crystallized from $CHCl_3$–MeOH or water to afford colorless needles of mp 214—216° (I). *Anal.* Calcd. for $C_{21}H_{24}O_{11}\cdot 1\frac{1}{2}H_2O$: C, 52.61; H, 5.68. Found: C, 52.15; H, 5.44.

Acetylation of I——Compound I (150 mg) in pyridine (0.5 ml) and acetic anhydride (1.5 ml) was allowed to stand for 18 hr at room temperature and the mixture was treated as usual to afford crystalline powder, which was chromatographed on a column of silica gel (50 g) with benzene–acetone (10: 1). The fraction of *Rf* 0.16 (TLC, benzene: acetone=10: 1) was crystallized from MeOH–petr-ether to afford colorless needles (II) of mp 130°. Yield: 37 mg. $[\alpha]_D^{16}=-9.2°$ ($c=1.11$, acetone). IR ν_{max} (cm^{-1}): 1750, 1230 (OAc). NMR (δ ppm, $CDCl_3$): 1.98 (s, 3H), 2.03 (s, 6H), 2.04 (s, 3H), 2.06 (s, 3H), 2.23 (s, 3H), 2.28 (s, 6H) (OAc×8), 2.72 (m, 2H, C_4–H_2), 3.88 (br, m, 1H, C_5''–H), 4.20 (m, 2H, C_6''–H_2), 5.00—5.40 (m, 6H, C_2–H, C_3–H, C_1''–H, C_2''–H, C_3''–H, C_4''–H), 6.40, 6.54 (2H, each d, $J=2.5$ Hz, C_6–H, C_8–H), 7.12—7.20 (3H, m, C_2'–H, C_5'–H, C_6'–H). MS: 788 (M^+), 398 (tetraacetyl catechin–AcOH), 356, 331. *Anal.* Calcd. for $C_{37}H_{40}O_{19}$: C, 56.34; H, 5.11. Found: C, 56.66; H, 5.06.

Methylation of I——The compound I (200 mg) in methanol was methylated with diazomethane to afford colorless needles (III) of mp 171—174° from methanol, after purification by silica gel column chromatography with $CHCl_3$–MeOH (5: 1). $[\alpha]_D^{19}=+19°$ ($c=1.0$, pyridine). NMR (d_5-pyridine): 2.92—3.54 (octet, 2H, $J_{AB}=16$ Hz, $J_{AX}=9$ Hz, $J_{BX}=6$ Hz, C_4–H_2), 3.68 (s, 3H), 3.73 (s, 6H) (OMe×3), 4.00—4.65 (br, 7H), 4.70—5.44 (br, 4H, OH×4), 5.04 (d, 1H, $J=8$ Hz, C_2–H), 5.50—5.90 (br, 2H, OH×2), 6.68, 6.84 (2H, each d, $J=2$ Hz, C_6–H, C_8–H), 6.96 (d, 1H, $J=8.5$ Hz, C_5'–H), 7.30 (m, 2H, C_2'–H, C_6'–H). *Anal.* Calcd. for $C_{24}H_{30}O_{11}\cdot H_2O$: C, 56.24; H, 6.29. Found: C, 56.14; H, 6.37.

Enzymatic Hydrolysis of III——To a solution of III (50 mg) in a small amount of methanol were added acetate buffer (pH 4.8, 50 ml) and 35 mg of emulsin (sigma). The mixture was incubated at 30° for 24 hr under stirring and then extracted with ethyl acetate. The ethyl acetate layer was concentrated to dryness *in vacuo* and the residue was chromatographed on a silica gel column with $CHCl_3$–MeOH (10: 1). The fraction of *Rf* 0.42 (TLC, $CHCl_3$: MeOH=20: 1) was crystallized from methanol to afford colorless plates (IV) of mp 261—264°. Ehrlich diazo reaction: positive (red-brown). Gibbs' test: negative. $[\alpha]_D^{19}=+38°$ ($c=1.0$, pyridine). UV λ_{max} (nm, log ε): 280 (3.59). NMR (d_5-pyridine): 2.97—3.57 (octet, 2H, $J_{AB}=16.3$ Hz, $J_{AX}=8.7$ Hz, $J_{BX}=5.8$ Hz, C_4–H_2), 3.63, 3.68, 3.71 (each s, 3H, OMe×3), 4.44 (m, 1H, C_3–H), 4.95 (br, 2H, OH×2), 5.03 (d, 1H, $J=9$ Hz, C_2–H), 6.51, 6.53, 6.64, 6.66 (AB_q. 2H, C_6–H, C_8–H), 6.88 (d, 1H, $J=9$ Hz, C_5'–H), 7.08—7.34 (m, 2H, C_2'–H, C_6'–H). MS (*m/e*, relative intensity): 332 (89, M^+), 180 (92), 165 (78), 153 (85), 152 (65), 151 (100), 137 (24). *Anal.* Calcd. for $C_{18}H_{20}O_6$: C, 65.05; H, 6.07. Found: C, 64.84; H, 6.15. IV was proved to be identical with 5,3′,4′-trimethyl-(+)-catechin[5] by the mixed fusion, IR and TLC.

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The aqueous layer which showed a spot of D-glucose at *Rf* 0.15 on a paper chromatogram (*n*-butanol: AcOH: H_2O=4: 1: 5) was concentrated and filtered through cation ion exchange resin. The filtrate was then concentrated to dryness to afford a substance, which gave D-glucose phenylosazone of mp 210.5—213° with phenylhydrazine (mixed fusion).

Methylation of IV——IV was methylated with diazomethane for 5 days to afford colorless needles (V) of mp 145—147°. *Rf*: 0.33 (TLC, acetone: ether: *n*-hexane=2: 5: 5). $[\alpha]_D^{24}=-15.0$ (c=1.00, $C_2H_2Cl_4$). NMR (d_6-acetone): 2.39—2.98 (octet, 2H, J_{AB}=16.5 Hz, J_{AX}=8.7 Hz, J_{BX}=5.7 Hz, C_4–H_2), 3.71 (s, 3H), 3.78 (s, 3H), 3.80 (s, 6H) (OMe×4), 4.00 (m, 1H, C_3–H), 4.16 (br, 1H, OH), 4.64 (d, 1H, J=8.8 Hz, C_2–H), 6.03, 6.12 (each d, 1H, J=2 Hz, C_6–H, C_8–H), 6.96—6.99 (m, 3H, C_2'–H, C_5'–H, C_6'–H). MS: 346 (M^+, 64), 180 (79), 167 (100), 166 (7), 165 (26), 151 (41), 137 (20). *Anal.* Calcd. for $C_{19}H_{22}O_6$: C, 65.88; H, 6.40. Found: C, 65.99; H, 6.47. V was proved to be identical with 5,7,3′,4′-tetramethyl-(+)-catechin[9] of mp 145—147°, ($[\alpha]_D^{16}=-13.1°$ (c=1.07, $C_2H_2Cl_4$); $[\alpha]_D^{16}=+7.3°$ (c=1.01, dimethylsulfoxide)), obtained by the methylation of (+)-catechin from gambir, by the mixed fusion, IR and TLC.

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Studies on Radioimmunoassay for 2,5-Dimethoxy-4-methylamphetamine

KUNISUKE NAGAMATSU, YASUMASA KIDO, and GORO URAKUBO

National Institute of Hygienic Sciences[1]

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A sensitive and specific method of radioimmunoassay for 2,5-dimethoxy-4-methylamphetamine (DOM) was developed, using anti-DOM antiserum obtained by immunizing guinea pig with DOM-glutaraldehyde-HSA conjugate and ^{125}I-N-succinyl-DOM-tyrosine methylester (^{125}I-DOM) as a labeled hapten.

DOM, ^{125}I-DOM and antibody came up to equilibrium over 15 hours at 4° of the incubation time in the radioimmunoassay system at pH 7.4 in phosphate buffer. Bound ^{125}I-DOM was precipitated with satd.$(NH_4)_2SO_4$ and the radioactivity of the bound labeled hapten was determined by γ-counting.

The displacement curve was linear when the percentage binding of ^{125}I-DOM was plotted against logarithmic increase of unlabeled DOM from 1 to 100 ng.

The antiserum showed less affinity for various phenylisopropylamine derivatives and biogenic amines and there was no interfering substance in a normal serum.

Keywords——radioimmunoassay; DOM; 2,5-dimethoxy-4-methylamphetamine; N-succinyl-DOM-tyrosine methylester; ^{125}I-N-succinyl-DOM-TME; hallucinogen; DOM-HSA

2,5-Dimethoxy-4-methylamphetamine (DOM) is a considerably potent hallucinogen which reveals the effect, lasting for about eight hours, with only a few mg.

The development of analytical method more sensitive than the conventional one of DOM was desired for pharmacokinetic studies.

Radioimmunoassay (RIA) technique using ^{3}H-labeled DOM has been reported[2] but was not sensitive enough to be satisfactory for the determination of DOM in biological fluids.

In the present paper, we wish to report a modified, more sensitive RIA method using ^{125}I-labeled hapten synthesized according to the route as shown in Chart 1.

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