Chem. Pharm. Bull. 33(10)4223—4227(1985)

Studies on the Neutral Constitutents of *Pachysandra terminalis* SIEB. *et* ZUCC. XI.[1] Occurrence of Pachysana-16,21-diene-3β,28-diol and Pachysan-16-ene-3β,28-diol, Novel Triterpene-diols with a New Skeleton Related to Friedelane

TOSHIO YOKOI,[a] TETSURO SHINGU,[a] SHIGETOSHI KADOTA,[b] and TOHRU KIKUCHI*,[b]

Faculty of Pharmaceutical Sciences, Kobe-Gakuin University,[a] Ikawadani-cho, Nishi-ku, Kobe 673, Japan and Research Institute for Wakan-Yaku (Oriental Medicines), Toyama Medical and Pharmaceutical University,[b] 2630 Sugitani, Toyama 930–01, Japan

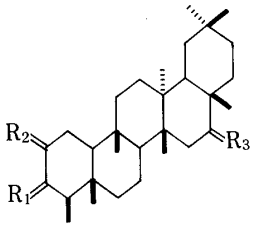
(Received February 14, 1985)

Pachysana-16,21-diene-3β,28-diol and pachysan-16-ene-3β,28-diol, new triterpene-diols having a 28-nor-16-methylfriedelane skeleton, were isolated from *Pachysandra terminalis* SIEB. *et* ZUCC. and their structures were elucidated. The name "pachysanane" is proposed for this new skeleton.

Keywords——pachysana-16,21-diene-3β,28-diol; pachysan-16-ene-3β,28-diol; triterpene; *Pachysandra terminalis*; 28-nor-16-methylfriedelane; pachysanane

In a series of studies on the neutral constituents of *Pachysandra terminalis* SIEB. *et* ZUCC., several new triterpenes such as pachysanediol-A (**1**), -B (**2a**), pachysonol (**3a**), pachysanetriol (**4**), pachysanedienol-A (**5a**), and -B (**6**) have been isolated and their structures established.[1,2] Among them, pachysanedienol-A (**5a**) and -B (**6**) were proved to have a 28-nor-16-methylfriedelane skeleton (**7**).[2a] However, the possibility remained that the skeleton (**7**) was formed by the migration of the 28-methyl group during the extraction and/or separation procedures, because 3-*O*-acetylpachysanediol-B (**2b**) and 3-*O*-acetyl-16-epipachysanediol-B (**2c**) were readily transformed to 28-nor-16-methylfriedelane derivatives by dehydration reaction of the 16-hydroxyl group.[3] In order to settle this problem, further examination of the neutral constituents of *Pachysandra terminalis* SIEB. *et* ZUCC. was carried out, and as a result, key substances (**8a** and **9a**) having the same skeleton with a 28-oxygenic function were isolated. This paper describes the structure elucidation of these novel compounds.

Compound **8a** is a minor triterpene of *P. terminalis* and was isolated as the diacetate (**8b**), $C_{34}H_{52}O_4$, mp 191—193.5 °C (see Experimental). The pure diol (**8a**), mp 246—247 °C, $[\alpha]_D$ +77.0°, ν_{max} 3600 and 3450 cm^{-1}, was obtained by alkaline hydrolysis of the diacetate (**8b**) and its molecular formula was determined to be $C_{30}H_{48}O_2$ by high-resolution mass spectral (MS) measurement. The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of the diacetate (**8b**) shows signals due to two acetyl groups (δ 2.051, 6H, s), six *tert*-methyl groups (δ 1.032—0.803), a *sec*-methyl group (δ 0.827, 3H, d, J=7.1 Hz), and a double bond (δ 6.312 and 5.538, each 1H, d, J=9.8 Hz). The ^{1}H-NMR pattern closely resembles that of pachysanedienol-A (**5b**)[2a] except for the appearance of characteristic signals ascribable to an acetoxymethylene group (δ 4.542 and 4.721, ABq, J=11.8 Hz) instead of the vinyl methyl signal in the latter compound (**5b**). The ultraviolet (UV) spectrum of **8b** exhibits absorption bands at 236 (sh), 243 (ε: 23600), and 252 (sh) nm, characteristic of a heteroannular conjugated diene system, and the spectral pattern is also similar to that of **5b**. These data suggested that the diacetate (**8b**) has the structure with an acetoxyl group attached at the C-28



1 : R_1 = α-OH, β-H ; R_2 = α-H, β-OH ; R_3 = H_2

2a : R_1 = α-OH, β-H ; R_2 = H_2 ; R_3 = α-OH, β-H

2b : R_1 = α-OAc, β-H ; R_2 = H_2 ; R_3 = α-OH, β-H

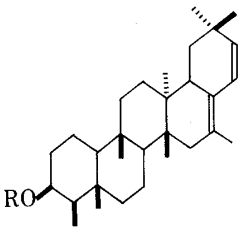
2c : R_1 = α-OAc, β-H ; R_2 = H_2 ; R_3 = α-H, β-OH

2d : R_1 = α-OAc, β-H ; R_2 = H_2 ; R_3 = α-OAc, β-H

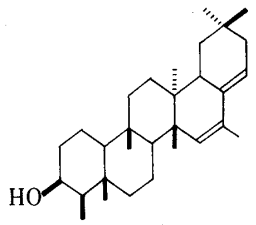
3a : R_1 = O ; R_2 = H_2 ; R_3 = α-OH, β-H

3b : R_1 = O ; R_2 = H_2 ; R_3 = α-OAc, β-H

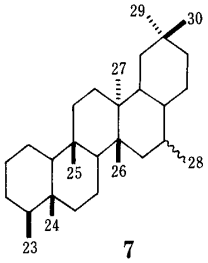
4 : R_1 = R_3 = α-OH, β-H ; R_2 = α-H, β-OH



5a : R = H
5b : R = Ac

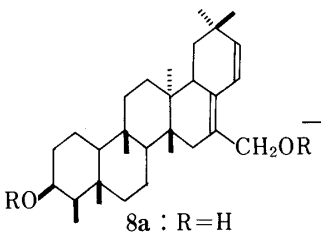


6

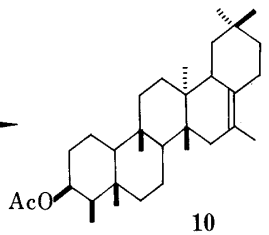


7

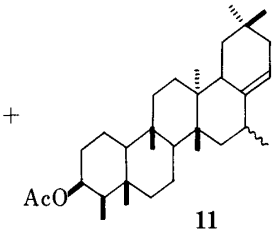
Chart 1



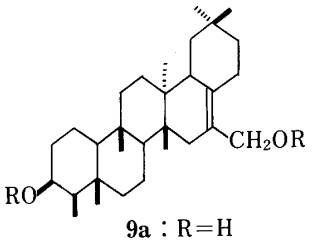
8a : R = H
8b : R = Ac



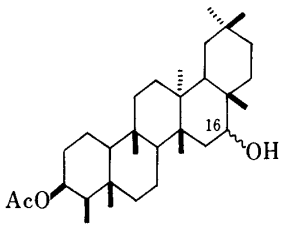
10



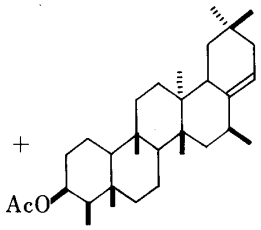
11



9a : R = H
9b : R = Ac



2b : 16β-OH
2c : 16α-OH



12

Chart 2

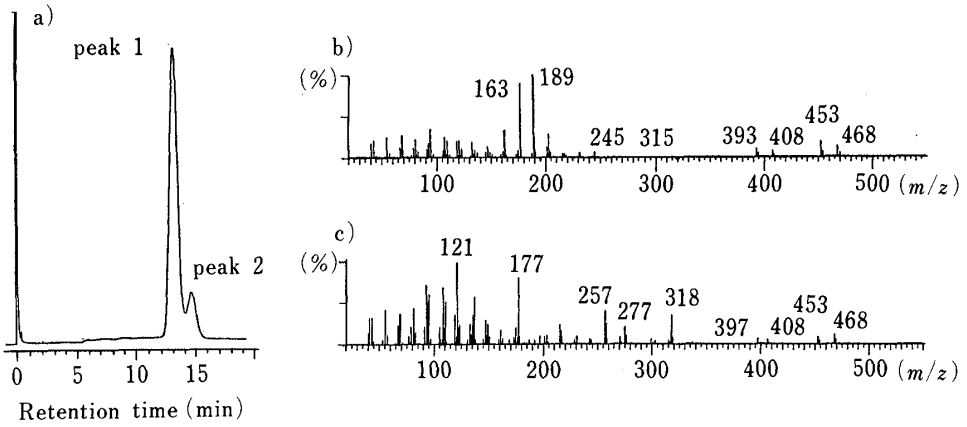
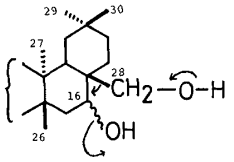


Fig. 1. GC-MS of Catalytic Reduction Products of **8b**

a) Gas chromatogram (column, 2% OV-17 (2 m); carrier gas, N_2; injection temp., 300 °C; column temp., 295 °C). b) MS of peak 1. c) MS of peak 2.



13

Chart 3

position in **5b**.

At this stage, allylic oxidation of **5b** with selenium dioxide was attempted to derive a compound with the presumed structure, but no oxidation product was obtained. Next, catalytic reduction of **8b** in the presence of 5% palladium–charcoal (Pd–C) was performed to give a mixture of two products (**10** and **11**) in a ratio of 85:15, as indicated by gas chromatographic (GC) analysis (Fig. 1).

The mass spectra obtained from peak 1 (**10**) and peak 2 (**11**) by the GC-MS method show the same molecular ion peak at *m/z* 468, corresponding to the composition $C_{32}H_{52}O_2$ (Fig. 1). Eventually, the mass spectra of these reduction products (**10** and **11**) were found to be indentical with those of **10** and **12**, respectively, which had been obtained by the dehydration reaction of 3-*O*-acetyl-16-epipachysanediol-B (**2c**)[3] and also by the catalytic reduction of 3-*O*-acetylpachysanedienol-A (**5b**).[2a]

Repeated column chromatography of the products on silver nitrate-impregnated silica gel led to the isolation of the major product (**10**), mp 215—225 °C, whose identity was confirmed by direct spectral comparisons with an authentic sample (**10**). Although the minor product could not be isolated because of the small amount available, it is probably identical with **12** or the 16-epimer.[4] It is plausible that the reduction of **8b** effected hydrogenolysis of the allylic acetoxyl group concurrently with hydrogenation to afford **10** and **11**. Thus, the structure of the diol was established to be **8a**.

The other minor triterpene (**9a**) was also isolated as the diacetate (**9b**), $C_{34}H_{54}O_4$, mp 208—209 °C (see Experimental). Alkaline hydrolysis of **9b** gave the diol (**9a**), amorphous solid, $[\alpha]_D$ +52.0°, ν_{max} 3600 and 3450 cm^{-1}, which was determined to have the molecular formula $C_{30}H_{50}O_2$ by high-resolution MS measurement. The ^{1}H-NMR spectrum of **9b** resembles that of **8b** except for the disappearance of two olefinic proton signals. Its UV spectrum exhibits no significant absorption band. These observations suggested that **9b** is a 21,22-saturated derivative of **8b**.

Catalytic reduction of **9b** in the presence of 5% Pd–C was carried out to afford only one

product, mp 225—230 °C, which was proved to be **10** by comparisons of the GC and ^{1}H-NMR characteristics with those of an authentic sample (**10**). Therefore, the structure of the original diol is represented by the formula **9a**.

It should be noted that **8a** and **9a** are not artifacts formed by 28-carbon migration, but naturally occurring compounds, since the precursor for such migration must have a hydroxyl group or its equivalent at the C-16 position as seen in **13** (Chart 3), and in this situation the 28-carbon unit would be preferentially eliminated in an acidic or alkaline environment.[5] Thus, pachysandienol-A (**5a**) and -B (**6**) are also naturally occurring compounds.

We propose the name "pachysanane" for the 28-nor-16-methylfriedelane skeleton. According to this system, our compounds, **8a** and **9a**, are pachysana-16,21-diene-3β,28-diol and pachysan-16-ene-3β,28-diol, respectively.

Experimental

Melting points were determined with a Kofler-type apparatus and are uncorrected. All specific rotations were measured in chloroform solution. GC analyses were done with a Hitachi 163 gas chromatograph under the following conditions: column, 2% OV-17 (2 m); injection temperature, 300 °C; column temperature, 295 °C; carrier gas, nitrogen (50 ml/min). High-resolution MS and GC-MS measurements were made with a JEOL JMS D-300 instrument. Infrared (IR) spectra were measured on a Hitachi 260-30 infrared spectrometer in chloroform solutions and UV spectra were taken with a Hitachi 323 UV spectrometer. ^{1}H-NMR spectra were taken on a Bruker AM-400 instrument in $CDCl_3$ solutions with tetramethylsilane as an internal standard, and chemical shifts are given in δ-values. Preparative thin layer chromatography (TLC) on argentic silica gel was done using Merck Kieselgel plates, impregnated with 10% $AgNO_3$ solution and dried at 120 °C for 20 h, and detection of adsorbed zones was done by spraying water. For extraction of substances from the Kieselgel, chloroform or methanol–chloroform mixture was used. Argentic silica gel (22.2%) for column chromatography was prepared according to Ghosh's description.[6]

Isolation of Pachysana-16,21-diene-3β,28-diol Diacetate (8b) and Pachysan-16-ene-3β,28-diol Diacetate (9b)—In a previous paper,[7] chromatographic separation of the neutral fraction from the MeOH extract of *P. terminalis* (dried, 120 kg), collected at Mt. Sasa-ga-mine, was described. The mother liquors from fractions 2—4 in that separation procedure (see Experimental in ref. 7) were used as the starting material in the present study. The combined mother liquors were concentrated *in vacuo* and the residue was chromatographed on alumina (4.6 kg, Brockmann II—III) with hexane, benzene–hexane (5 : 95, 1 : 9, 3 : 7, and 1 : 1), benzene, ether–benzene (2 : 8), and MeOH–benzene (3 : 7) as eluents. The benzene eluate (*ca.* 28 g) was treated with CH_2Cl_2–ether, giving a crystalline material (7 g) which on crystallization from ether–CH_2Cl_2 afforded pachysanediol-B (**2a**) (crude crystals, 3.2 g) and a mixture of pachysonol (**3a**) and pachysanediol-B (**2a**) (0.76 g). Further treatment of the mother liquors with CH_2Cl_2–ether gave a precipitate (3 g), which was separated into a less soluble portion (0.97 g) and a more soluble one (*ca.* 2 g). The former portion was chromatographed on silica gel (160 g, Mallinckrodt CC-7) with chloroform and each fraction was monitored by TLC. Earlier fractions gave an additional crop of pachysonol (**3a**) (*ca.* 50 mg). Later fractions, corresponding to **2a** in TLC, were combined (*ca.* 250 mg) and acetylated with Ac_2O and pyridine (each 2.5 ml). Usual work-up afforded an acetate mixture (250 mg), which was subjected to preparative TLC on silica gel (developed with benzene). The more polar fraction (180 mg) was further separated by repeated preparative TLC on $AgNO_3$–silica gel (developed with 1 : 1 benzene–chloroform); the substance from the lower zone (56 mg) was recrystallized from CH_2Cl_2–MeOH to give pachysana-16,21-diene-3β,28-diol diacetate (**8b**) (38 mg), colorless needles, mp 191—193.5 °C. $[\alpha]_D^{20}$ +89.7° (c=0.8). MS m/z: 524 (M^+), 464 (M^+ −AcOH), 449, 404 (M^+ −2AcOH), 389. High-resolution MS m/z: Found 524.3864 (M^+), Calcd for $C_{34}H_{52}O_4$ 524.3868; Found 464.3672, Calcd for $C_{32}H_{48}O_2$ 464.3655; Found 449.3449, Calcd for $C_{31}H_{45}O_2$ 449.3419; Found 404.3460, Calcd for $C_{30}H_{44}$ 404.3443; Found 389.3249, Calcd for $C_{29}H_{41}$ 389.3208. UV (EtOH) λ_{max} nm: 236 (sh), 243 (ε: 23600), and 252 (sh). IR ν_{max} cm^{-1}: 1720 and 1260 (OAc). ^{1}H-NMR (400 MHz) δ: 0.803 (6H, s, 2 × *tert*-CH_3), 0.827 (3H, d, J=7.1 Hz, *sec*-CH_3), 0.884, 0.946, 1.024, 1.032 (each 3H, s, *tert*-CH_3), 2.051 (6H, s, Ac), 4.542, 4.721 (2H, ABq, J=11.9 Hz, –C$\underline{H}_2$–OAc), 4.900 (1H, m, >C$\underline{H}$–OAc), 5.538, 6.312 (each 1H, d, J=9.8 Hz, $\underline{H}$>C=C<$\underline{H}$).

On the other hand, the substance from the upper zone was again separated by preparative TLC (Merck Kieselgel PF_{254}) to give a small amount of pachysanediol-B diacetate (**2d**) and pachysan-16-ene-3β,28-diol diacetate (**9b**) (30 mg), colorless needles (from CH_2Cl_2–MeOH), mp 208—209 °C. $[\alpha]_D^{18}$ +47.4° (c=1.0). MS m/z: 526 (M^+, very weak), 466 (M^+ −AcOH), 451, 406 (M^+ −2AcOH), 391. High-resolution MS m/z: Found 466.3800 (M^+ −AcOH), Calcd for $C_{32}H_{50}O_2$ 466.3810; Found 451.3592, Calcd for $C_{31}H_{47}O_2$ 451.3576; Found 406.3637, Calcd for $C_{30}H_{46}$ 406.3599; Found 391.3366, Calcd for $C_{29}H_{43}$ 391.3365. IR ν_{max} cm^{-1}: 1715 and 1260 (OAc). ^{1}H-NMR (400 MHz) δ: 0.738, 0.793, 0.870, 0.923, 0.936, 0.955 (each 3H, s, *tert*-CH_3), 0.821 (3H, d, J=6.8 Hz, *sec*-CH_3), 2.044, 2.048 (each 3H, s, Ac), 4.513, 4.563 (2H, ABq, J=11.8 Hz, –C$\underline{H}_2$–OAc), 4.895 (1H, m, >C$\underline{H}$–OAc).

Pachysana-16,21-diene-3β,28-diol (8a)——Mixture of the diacetate (**8b**) (20 mg) and 5% KOH–MeOH (10 ml) was refluxed for 5 h. The reaction mixture was concentrated *in vacuo*, diluted with water, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water, dried ($MgSO_4$), and concentrated to give a crystalline residue (16 mg). The residue was recrystallized from $CHCl_3$–MeOH to afford colorless needles (**8a**) (10 mg), mp 246—247 °C. $[\alpha]_D^{20}$ +77.0° ($c=0.8$). MS *m/z*: 440 (M^+, base peak), 422 ($M^+ - H_2O$), 407. High-resolution MS *m/z*; Found 440.3631 (M^+), Calcd for $C_{30}H_{48}O_2$ 440.3651; Found 422.3506, Calcd for $C_{30}H_{46}O$ 422.3546; Found 407.3330, Calcd for $C_{29}H_{43}O$ 407.3314. UV (EtOH) λ_{max} nm: 236 (sh), 244 (ε: 19000), 254 (sh). IR ν_{max} cm^{-1}: 3600 and 3450 (OH). ^{1}H-NMR (400 MHz) δ: 0.803, 0.807, 0.883, 0.981, 1.029, 1.031 (each 3H, s, *tert*-CH_3), 0.950 (3H, d, $J=7.2$ Hz, *sec*-CH_3), 3.749 (1H, m, >C<u>H</u>–OH), 4.076, 4.268 (2H, ABq, $J=11.6$ Hz, –C<u>H</u>$_2$–OH), 5.522, 6.340 (each 1H, d, $J=10.0$ Hz, <u>H</u>>C=C<<u>H</u>).

Catalytic Reduction of Pachysana-16,21-diene-3β,28-diol Diacetate (8b)——Pachysana-16,21-diene-3β,28-diol diacetate (**8b**) (5 mg) was hydrogenated over 5% Pd–C (20 mg) in MeOH (5 ml) at room temperature and atmospheric pressure for 5 h. The catalyst was filtered off and the filtrate was concentrated *in vacuo* to afford a crystalline residue (4 mg, roughly 85 : 15 mixture of **10** and **11**, as estimated by GC analysis). A part of this residue was subjected to GC-MS analysis, and the result is shown in Fig. 1. On the other hand, the rest of the residue was repeatedly chromatographed on an $AgNO_3$–SiO_2 column to afford the major product (**10**) (1 mg), mp 215—225 °C. High-resolution MS *m/z*: Found 468.3981 (M^+), Calcd for $C_{32}H_{52}O_2$ 468.3967. This product was shown to be identical with an authentic sample (**10**) by GC and NMR comparisons.

Pachysan-16-ene-3β,28-diol (9a)——A mixture of pachysan-16-ene-3β,28-diol diacetate (**9b**) (13 mg) and 3% KOH–MeOH (10 ml) was refluxed for 4 h. The reaction mixture was concentrated *in vacuo*, diluted with water, and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with water, dried ($MgSO_4$), and concentrated to give an amorphous solid (**9a**) (8 mg). $[\alpha]_D^{18}$ +52.0° ($c=1.0$). MS *m/z*: 442 (M^+), 424, 409, 406, 391. High-resolution MS *m/z*: Found 442.3799 (M^+), Calcd for $C_{30}H_{50}O_2$ 442.3810; Found 424.3657, Calcd for $C_{30}H_{48}O$ 424.3703; Found 409.3422, Calcd for $C_{29}H_{45}O$ 409.3469; Found 406.3591, Calcd for $C_{30}H_{46}$ 406.3598; Found 391.3368, Calcd for $C_{29}H_{43}$ 391.3365. IR ν_{max} cm^{-1}: 3600 and 3450 (OH). ^{1}H-NMR (400 MHz) δ: 0.743, 0.800, 0.867, 0.927, 0.960, 0.973 (each 3H, s, *tert*-CH_3), 0.945 (3H, d, $J=7.3$ Hz, *sec*-CH_3), 3.743 (1H, br s, >C<u>H</u>–OH), 4.004, 4.151 (2H, ABq, $J=11.5$ Hz, –C<u>H</u>$_2$–OH).

Catalytic Reduction of Pachysan-16-ene-3β,28-diol Diacetate (9b)——Pachysan-16-ene-3β,28-diol diacetate (**9b**) (1.5 mg) was hydrogenated over 5% Pd–C (14 mg) in EtOH (8 ml) at room temperature and atmospheric pressure for 4 h. The catalyst was filtered off and the filtrate was concentrated *in vacuo* to afford a crystalline residue (1.5 mg). The residue was subjected to preparative TLC (Merck Kieselgel PF_{254}) to give a pure sample (**10**) (0.8 mg), mp 225—230 °C. High-resolution MS *m/z*: Found 468.3990 (M^+), Calcd for $C_{32}H_{52}O_2$ 468.3967; Found 453.3715, Calcd for $C_{31}H_{49}O$ 453.3731; Found 408.3757, Calcd for $C_{30}H_{48}$ 408.3756; Found 393.3481, Calcd for $C_{29}H_{45}$ 393.3520; Found 189.1659, Calcd for $C_{14}H_{21}$ 189.1644; Found 177.1671, Calcd for $C_{13}H_{21}$ 177.1643. This compound was identified as **10** by GC, ^{1}H-NMR, and MS comparisons with an authentic sample.

References and Notes

1) Part X: T. Yokoi and T. Kikuchi, *Yakugaku Zasshi*, **105**, 690 (1985).
2) *a*) T. Kikuchi, T. Yokoi, T. Shingu, and M. Niwa, *Chem. Pharm. Bull.*, **29**, 2531 (1981); *b*) T. Kikuchi and T. Toyoda, *ibid.*, **19**, 753 (1971); *c*) T. Kikuchi, M. Takayama, T. Toyoda, M. Arimoto, and M. Niwa, *ibid.*, **21**, 2243 (1973); *d*) T. Kikuchi and M. Niwa, *Yakugaku Zasshi*, **93**, 1378 (1973).
3) T. Kikuchi, M. Niwa, T. Yokoi, and S. Kadota, *Chem. Pharm. Bull.*, **29**, 1819 (1981).
4) The retention time of peak 2 in GC (Fig. 1) differs slightly from that of **12**, although no separation of the peaks was observed in co-injection experiments.
5) An example of this 1,3-diol fragmentation is the reverse Prins reaction; *cf.* C. A. Grob and P. W. Schiess, *Angew. Chem.*, **79**, 1 (1967).
6) A. Ghosh, M. Hoque, and J. Dutta, *J. Chromatogr.*, **69**, 207 (1972).
7) T. Kikuchi, T. Toyoda, M. Arimoto, M. Takayama, and M. Yamano, *Yakugaku Zasshi*, **89**, 1358 (1969).