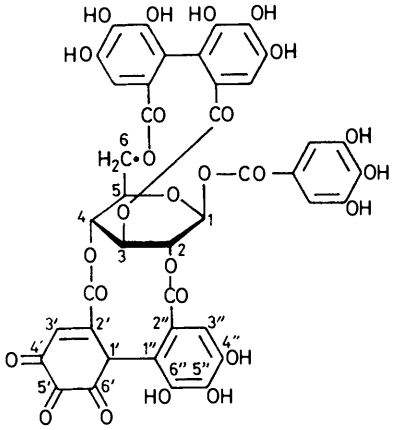
Constituents of *Geranium thunbergii* Sieb. *et* Zucc. Part 12.[1] Hydrated Stereostructure and Equilibration of Geraniin

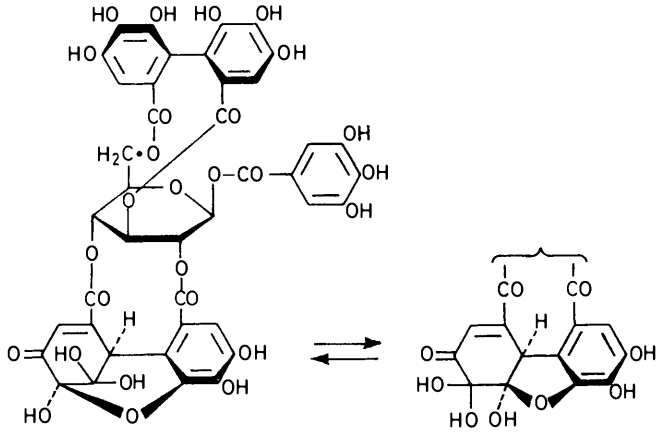
By **Takuo Okuda,* Takashi Yoshida,** and **Tsutomu Hatano,** Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700, Japan

The structure of geraniin is (1), in which the cyclohexenetrione moiety is attached to O-4 of glucose. It forms a hydrated six-membered hemiacetal-ring structure (1a) in the crystalline state, and an equilibrium mixture of (1a) and a hydrated five-membered hemiacetal-ring structure (1b) upon the mutarotation. The absolute configurations are also represented by (1a) and (1b).

GERANIIN (1) is the main tannin of plants of several *Geranium* species, and also of Euphorbiaceae.[2] This ellagitannin was isolated from *Geranium thunbergii* SIEB. *et* ZUCC. in an attempt to obtain the active principle of this medicinal plant, which is one of the most popular folk medicines and also is an official antidiarrheic in Japan.[3] Upon hydrolysis of geraniin, gallic acid, hexahydroxydiphenic acid, corilagin, and brevifolincarboxylic acid were produced.[4] The condensation of geraniin with *o*-phenylenediamine yielded ' phenazine A ' (2) which was converted to ' phenazine B ' (3) in acidic solution.[4] The latter product gave corilagin (4) and ' phenazine C ' (5) upon hydrolysis.[4] Based on these results combined with spectral evidence, the structure (1), or its isomer in which the orientation of the dehydrohexahydroxydiphenoyl (DHHDP) group is reversed, was elucidated for geraniin. The latter structure was temporarily assigned to geraniin in a preceding paper of this series.[4] Hydration of two of the three ketone carbonyls in geraniin to form *gem*-diols was also presumed.[5] In the present study, we present evidence which indicates that the structure of geraniin in the crystals is (1a), and also that it forms an equilibrium mixture (1a $\rightleftharpoons$ 1b) in aqueous solution.[6]



(1)

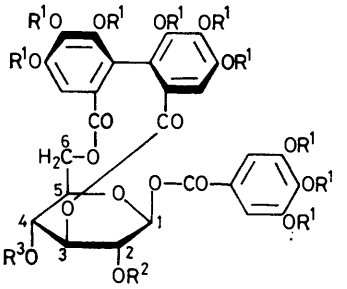


(1a) (1b)

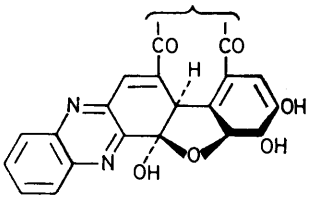
RESULTS AND DISCUSSION

It has now been found that of the two ester groups linking the DHHDP group with O-2 and O-4 of glucose in geraniin, an ester group links the phenyl ring with O-2 of glucose and another ester group links the cyclohexenetrione ring and O-4 of glucose. Methylation of ' phenazine B ' (3) with wet diazomethane solution gave the tetradecamethyl derivative (6), $C_{61}H_{60}N_2O_{25}$, which showed mass-spectral peaks at *m/e* 1 009 [M^+ H — tri-*O*-methylgallic acid]$^+$ and 479 {base peak, [(11) + H]$^+$} (chemical ionisation). Derivative (6) yielded the monoacetate (7), $C_{63}H_{62}N_2O_{26}$ [^{1}H n.m.r. spectrum ($CDCl_3$): δ 2.36 (acetyl)] The ^{1}H n.m.r. spectra of (6) and (7) indicate that the ester linkage at O-2 of the glucose moiety in (3) was cleaved upon the formation of (6), since H-2, which was shifted upfield and hidden by other protons in (6), shifts downfield to δ 5.32 (coupled with H-1 at δ 6.56) in (7). Permethylation of (6) gave the pentadecamethyl derivative (8) which yielded upon methanolysis 2-*O*-methylglucose, which supports the assignment of the free hydroxy-group at C-2 in (6). The same methyl derivative (6) was obtained upon methylation of (2) with diazomethane. Acetylation of (2) and (3) also gave the same acetate (9). When methylation of (3) was carried out with dry diazomethane, the tridecamethyl derivative (10), in which all the ester groups in (3) are retained, was produced. The methylation of (2) with dry diazomethane also produced (10).

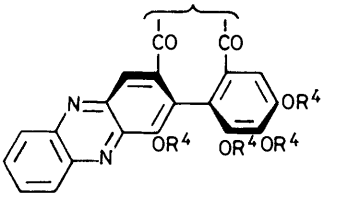
Upon hydrolysis of (7) in refluxing 10% HCl, the phenylphenazine residue (11) was isolated by prepara-



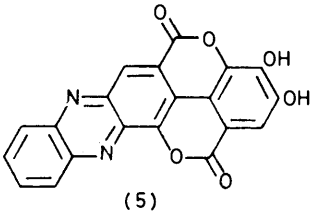
(2) R^1 = H, R^2–R^3 = (A)
(3) R^1 = H, R^2–R^3 = (B)
(4) R^1 = R^2 = R^3 = H
(6) R^1 = Me, R^2 = H, R^3 = (C)
(7) R^1 = Me, R^2 = Ac, R^3 = (C)
(8) R^1 = R^2 = Me, R^3 = (C)
(9) R^1 = Ac, R^2–R^3 = (D)
(10) R^1 = Me, R^2–R^3 = (E)
(14) R^1 = Me, R^2 = R^3 = H



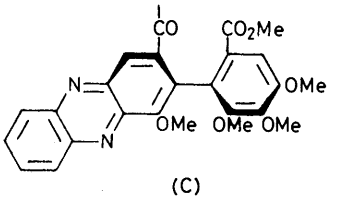
(A)



(B) R^4 = H
(D) R^4 = Ac
(E) R^4 = Me



(5)



(C)

tive t.l.c. It yielded, upon methylation with diazomethane, the dimethyl ester (12) which was identified with methyl 4-methoxy-3-(4,5,6-trimethoxy-2-methoxycarbonylphenyl)phenazine-2-carboxylate.[4] The same ester was also obtained upon methanolysis of (10).

The location of the free carboxy-group in (11) was determined to be at C-2′* by comparison of the mass spectra of the phenazine derivatives (Table). The ion peaks

TABLE

Ions in the mass spectra of phenazine derivatives, (11), (12), (12′), and (13) *

Compound	M^+	(%)	(a)	(%)	(*b*)	(%)
(11)	478	(59)	267	(3)	239	(6)
(12)	492	(43)	281	(8)	239	(5)
(12′)	498	(64)	284	(5)	242	(5)
(13)	464	(80)	267	(4)	225	(4)

* Temperature of ion source 250 °C, ion accelerating voltage 3.5 kV, ionizing potential 70 eV, trap current 60 μA.

which are attributable to the ion (a), which arises *via* the rearrangement[7] upon cleavage of the diphenyl moiety, are at *m/e* 267 for (11) and the dicarboxylic acid (13), and at *m/e* 281 for the dimethyl ester (12). The assignment of ion (a) was confirmed by the fragment ion at *m/e* 284 in the mass spectrum of the deuteriated derivative (12′).

The tetradecamethyl derivative of (3) is therefore

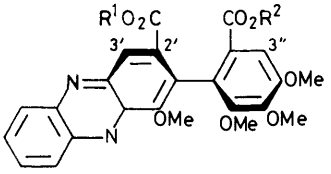
* This numbering is based on that of (1).

formulated as (6), and the ester linkages in ' phenazine B ' are shown as in (3). These results indicate that the ester linkages at C-2 and C-4 of the glucose moiety in geraniin are as in structure (1).

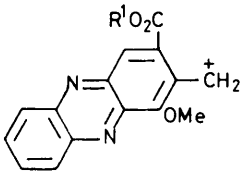
Crystalline geraniin, $C_{41}H_{28}O_{27}\cdot xH_2O$, exhibits mutarotation upon dissolution in acetone—water {$[\alpha]_D^{19}$ −148 → −132°, 5 h; acetone–water (9 : 1 v/v); $x = 7$}. Crystalline geraniin was recovered by evaporation of the solution followed by recrystallization. The structural transformation upon this equilibration between the two structures, (1a) and (1b), was demonstrated by the ^{1}H n.m.r. and ^{13}C n.m.r. spectra measured in [$^2H_6$]acetone containing D_2O. In the ^{1}H n.m.r. spectra, the singlets of the methine and vinyl protons, which were at δ 5.16 and 6.56 in [$^2H_6$]acetone before the mutarotation (1a), decreased in peak area during mutarotation. Two newly formed mutually coupled doublets (J 1.5 Hz) at δ 4.72 and 6.26 increased in peak area simultaneously, until the ratio of the peak areas of the original peaks and their counterparts [due to (1b)] was *ca.* 1 : 1. The occurrence of (1b) upon equilibration is also observed for the aromatic protons, as the peaks of original aromatic protons at δ 6.71, 7.13, 7.22, and 7.25 decreased their peak areas while new peaks at δ 6.69, 7.08, 7.20, and 7.28 increased their peak areas.

The completely proton-decoupled ^{13}C n.m.r. spectrum

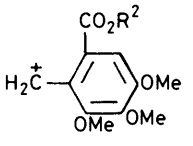
measured a short time after dissolution of crystalline geraniin in [$^2H_6$]acetone showed a single peak for each carbon. The assignment of each carbon peak was based on comparison with the peaks of corilagin.[1] The carbon signals of the DHHDP group in geraniin were assigned to the residual peaks after subtracting the peaks of corilagin from those of geraniin.[1]



(11) R^1=H, R^2=Me
(12) R^1=R^2=Me
(12') R^1=R^2=CD
(13) R^1=R^2=H



(a)
R^1=H (*m/e* 267)
R^1=Me (*m/e* 281)
R^1=CD_3 (*m/e* 284)



(b)
R^2=H (*m/e* 225)
R^2=Me (*m/e* 239)
R^2=CD_3 (*m/e* 242)

Upon equilibration, each carbon in the ^{13}C n.m.r. spectrum showed two peaks, in a *ca.* 1 : 1 ratio. The peak of the methine carbon at δ 46.2 in (1a) was accompanied by another peak at δ 51.9 due to (1b) after equilibration. Compound (1a) showed only one of the three ketonic carbons at δ 191.8, and two peaks in the region for hydrated ketones or hemiacetals (δ 92.5 and 96.3). Upon equilibration, another ketonic carbon peak at δ 194.8, and two additional peaks in the region of hemiacetal carbons (δ 92.3 and 108.9) appeared.

The gated-decoupling technique showed two doublets (δ 92.5 and 108.9) and two triplets (δ 92.3 and 96.3 p.p.m.) which were singlets in the spectrum obtained by the off-resonance technique. As the ^{13}C n.m.r. spectrum of ninhydrin obtained for comparison with that of geraniin showed no coupling between the hydrogen and the carbon of the *gem*-diol group, the couplings described above are attributable to those of ^{13}C–C–H and ^{13}C–C–C–H on the cyclohexene ring. Therefore the doublets can be assigned to C-6', and the triplets to C-5'.

These shifts in the ^{13}C n.m.r. spectra, and the observation in the ^1H n.m.r. spectra that the methine proton is not substituted by deuterium upon the equilibration of dried geraniin in [$^2H_6$]acetone containing D_2O, indicate that the equilibration of geraniin is not due to the epimerization at C-1', which was presumed previously.[5]

The large downfield shift of the C-6' peak from δ 92.5 to 108.9 upon equilibration may be attributed either to formation of an ether linkage, or a transformation analogous to that from pyranose to furanose.[8] The C-6' peak of (2) which is produced by condensation of *o*-phenylenediamine with geraniin at C-4' and C-5',[4] is at δ 106.6. The analogy of the chemical shift of C-6' in (2) in which formation of a six-membered hemiacetal ring is impossible, to the peak of the same carbon in the (1b)-form of geraniin, indicates the formation of a five-membered hemiacetal ring in the mutarotated geraniin as shown by (1b). Although (2) has a ketone group at C-6', which will be enolisable if it is free, the formation of the five-membered hemiacetal ring rationalizes the fairly stable character of (2) which does not immediately enolise to (3).

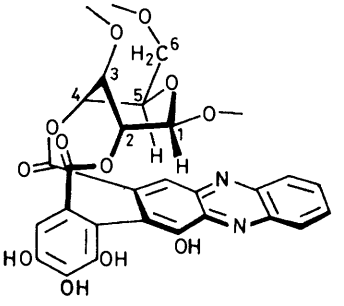
An allylic coupling is observed between H-1' and H-3' in the ^1H n.m.r. spectrum of (2) and also in the (1b)-form of geraniin (*J* 1.5 Hz). This coupling is in accord with the conformation of the cyclohexenetrione ring, which is induced by the ring closure to a five-membered hemiacetal in (1b) and (2), in which the angle between the two bonds, C-1'–H-1' and C-3'–H-3' is *ca.* 90°, and gives further support to both structures (2) and (1b).

Comparison of the ^{13}C n.m.r. peaks of the DHHDP moiety in the two forms of geraniin indicates formation of a six-membered hemiacetal ring as in (1a), or presence of two *gem*-diol groups in the a-form of geraniin. Between these two structures, the former has been supported by the deuterium-induced differential isotope shift (DIS) measurement. As this technique was originally developed for the analysis of carbohydrates,[9] in the preliminary experiment we tested the applicability of this technique to phenolic compounds by measuring phenols of lower molecular weights such as arbutin and corilagin. The result of this experiment showed that the aromatic carbon attached to a free hydroxy-group is clearly differentiated from that attached to an ether oxygen, as the former showed a dual peak (separation 0.1—0.25 p.p.m.) while the latter showed a single peak.

Among the phenolic carbons of geraniin, only C-6'' exhibited a single peak in the (1a) and (1b) forms of geraniin, at δ 143.4 and 147.3, respectively. All the other phenolic carbons exhibited dual peaks; e.g. for C-4'', the peak separation was 0.13 p.p.m. (in [$^2H_6$]-acetone–H_2O and D_2O, 1 : 1 v/v). This result shows that both the (1a) and (1b) forms of geraniin have a hemiacetal ring instead of two *gem*-diol groups, and therefore that a six-membered hemiacetal ring is present in the (1a) form of geraniin. The peaks of H-1' and H-3' in the ^1H n.m.r. spectrum of the (1a) form of geraniin, which are sharp singlets, also give support to the six-membered hemiacetal ring, since the bonds C-1'–H-1' and C-3'–H-3' in this structure are almost in the same plane. The upfield shift of the C-5' peak in the ^{13}C n.m.r. spectra (δ 96.3 ⟶ 92.3 p.p.m.) upon the equilibration of geraniin

is also in accord with the structural transformation of (1a) to (1b).

As for the atropisomerism at the hexahydroxydiphenoyl (HHDP) group in corilagin, no unambiguous evidence has been presented at the present time. The only paper which proposed the *S*-configuration for this diphenyl group was based on an attempted extension of the amide rule, which was originally applied to glyconic acids.[10] In the present investigations, nona-*O*-methyl-

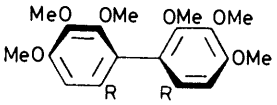


(3a)

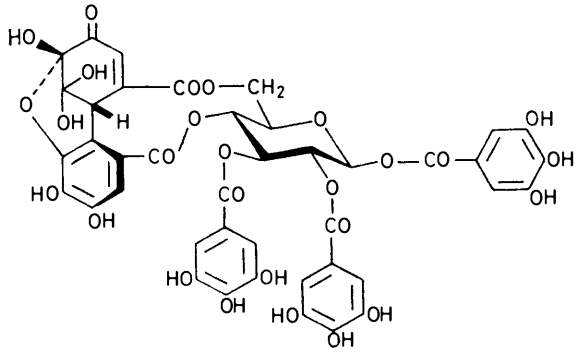
corilagin (14) was methanolysed to give dimethyl hexamethoxydiphenate (15), ($[\alpha]_D^{25}$ +21°, $CHCl_3$) which was then reduced to dihydroxymethylhexamethoxydiphenyl (16) ($[\alpha]_{240}$ +2 800°, MeOH). The optical activities of these products were found identical with the corresponding compounds derived from schizandrin, for which the absolute configuration at the diphenyl group had been determined to be *R*.[11] The determination of the *R*-configuration at the HHDP moiety in corilagin also established the absolute configuration at the HHDP moiety in geraniin to be *R*, since corilagin is produced by hydrolysis of geraniin.[4]

The absolute configuration at the phenylphenazine moiety in (3) is regarded as *R*, based on the following data. A marked upfield shift for H-1 (δ 6.62⟶6.14) and downfield shift for H-5 (δ 4.39⟶4.99) in the glucose moiety are observed in the ^{1}H n.m.r. spectrum upon the transformation of (2) to (3). These shifts should be attributed to the shielding and deshielding effects by the phenylphenazine moiety in the conformation (3a) of (3). This conformation requires the absolute configuration at the phenylphenazine moiety to be *R*. The configuration of H-1′ in (2), and accordingly in geraniin also, is then assigned α since the *R* configuration of the phenylphenazine group in (3) is regarded as due to this stereostructure of the precursor. Further evidence is presented by the dextrorotatory dimethyl ester (12) ($[\alpha]_D^{23}$ +37°, *c* 1.2 in $CHCl_3$) obtained by methanolysis of (6), and also by methylation of (11). An optical antipode of (12) was reported to be produced from isoterchebin [12,13] which has the DHHDP group on O-4 and O-6 of D-glucopyranose in its revised structure (17).[13] Isoterchebin was reduced with sodium dithionite,[14] and also by catalytic hydrogenation, and the products of both experiments were identified as 1,2,3-tri-*O*-galloyl-4,6-*O*-hexahydroxydiphenoyl-β-D-glucopyranose,[15] for which the configuration of the diphenyl group was recently established to be *S*.[13] These results indicate the *S*-configuration at the methine carbon in isoterchebin, and also the *S*-configuration of the optical antipode of (12). These results therefore support the *R*-configuration at the phenylphenazine moiety of (3), and the *R*-configuration at the methine carbon in geraniin.

The hemiacetal hydroxy-group in (2) and in (1a) and (1b) should also be α-orientated, since the hemiacetal rings having α-H at C-1′ do not allow β-orientation of the hemiacetal hydroxy-group.



(15) R = CO_2Me
(16) R = CH_2OH



(17)

These data indicate the absolute stereostructure of geraniin before mutarotation to be (1a) and that of equilibrated geraniin to be (1a⇄1b).

EXPERIMENTAL

^{1}H N.m.r. spectra were obtained at 90 MHz with Hitachi R22 instrument, and ^{13}C n.m.r. spectra were mostly measured at 22.6 MHz with an R22 FTS instrument; chemical shifts were referred to internal $SiMe_4$. Electron-impact mass spectra were obtained with Shimadzu-LKB 9 000, and chemical ionization mass spectra (2-methylpropane) were recorded with 9 000B instruments using direct-inlet systems. Optical rotations at 589 nm were measured on a JASCO DIP-4 Digital Polarimeter, and o.r.d. spectra were measured with a JASCO ORD/UV-5 Spectrometer. T.l.c. was carried out on Kieselgel PF_{254} (Merck) in the systems (A) benzene–chloroform–acetone (3 : 1 : 1, v/v), (B) chloroform–acetone (3 : 1, v/v), and (C) ligroin–acetone–dichloromethane (6 : 1 : 3, v/v).

^{13}C N.m.r. *Shifts of the DHHDP Group in Geraniin.*—Compound (1a), δ 46.2(1′), 154.5 (2′), 128.6 (3′), 191.8 (4′),

96.3 (5′), 92.5 (6′), 115.3 (1″), 119.4 (2″), 113.5 (3″), 144.6 (4″), 139.0 (5″), and 143.4 (6″): compound (1b), δ 51.9 (1′), 149.2 (2′), 125.0 (3′), 194.8 (4′), 92.3 (5′), 108.9 (6′), and 147.3 (6″).

Methylation of 'Phenazine B' (3).—(*a*) Methylation of (3) (200 mg) in ethanol, with a solution of diazomethane in ether which was not dried, gave (6) as a yellow amorphous powder. Preparative t.l.c. [R_F 0.52, solvent (A)] followed by crystallization from dichloromethane–ethanol afforded yellow *crystals*, m.p. 156—158 °C (120 mg) (Found: C, 60.0; H, 4.85; N, 2.05. $C_{61}H_{60}N_2O_{25}$ requires C, 60.0; H, 4.95; N, 2.29%). The ^{1}H n.m.r. spectrum [$(CD_3)_2CO$] showed the presence of fourteen methoxy-groups at δ3.28—4.00, and absence of the glucose H-2 in the region of acylated-carbon protons; c.i.-m.s., *m/e* 1 009 [M^+H − tri-*O*-methylgallic acid]$^+$, 479 [(11) + H]$^+$, and 213 [tri-*O*-methylgallic acid + H]$^+$. This product was not detected [t.l.c., solvent (A)] when the methylation was carried out with a solution of diazomethane in ether which had been dried over potassium hydroxide.

(*b*) Compound (3) (50 mg) in dry acetone (2 ml) was methylated for 2 h with a diazomethane solution, in ether which had been dried over potassium hydroxide. The crude crystalline residue obtained upon evaporation of ether was recrystallized from dichloromethane–ethanol to give (10) as fine yellow needles, m.p. 292 °C (decomp.) (30 mg) (Found: C, 60.3; H, 4.95; N, 2.1. $C_{60}H_{56}N_2O_{24}$ requires C, 60.61; H, 4.75; N, 2.36%).

Methylation of (2).—Methylation of (2) (200 mg) with diazomethane (not dried) was carried out in the same way as that of (3). The product was purified by preparative t.l.c., and identified with (6) by i.r. and ^{1}H n.m.r. spectra, and t.l.c.

Acetylation of the Tetradecamethyl Derivative of (3).—The methylated derivative (6) (50 mg) was acetylated with acetic anhydride (2 ml) and pyridine (2 ml) overnight, and after the usual treatment, the product [(7), R_F 0.46 (A)] was isolated by preparative t.l.c. [solvent (B)] as a yellow amorphous *powder* (50 mg) (Found: C, 59.0; H, 5.05; N, 2.05. $C_{63}H_{62}N_2O_{26}\cdot H_2O$ requires C, 59.06; H, 5.03; N, 2.18%); δ($CDCl_3$) 2.36, (s, Ac) and 5.32 (m, glucose H-2).

Permethylation of the Tetradecamethyl Derivative (6).—The methylated derivative (6) (31.2 mg) was permethylated in chloroform with diazomethane (ether, 30 ml) and boron trifluoride–ether (ca. 0.2 ml) for 30 min. After evaporating the solvent, the residue was extracted with chloroform. The main *product* (8) which moved faster (R_F 0.70) than (6) (R_F 0.52) on t.l.c. was isolated by preparative t.l.c. (6.9 mg) and crystallized from dichloromethane–ethanol, m.p. 173—175 °C (Found: C, 60.05; H, 4.9; N, 1.95. $C_{62}H_{62}N_2O_{25}$ requires C, 60.29; H, 5.06; N, 2.27%). Compound (8) was methanolysed with 0.2% sodium methoxide in methanol (5 ml) for 1.5 h. After neutralization and evaporation, the residue was distributed in dichloromethane–water (1 : 1 v/v), and the aqueous layer was evaporated. G.l.c. (2.5% OV-1, 170 °C) of the trimethylsilylated residue showed two peaks corresponding to the α- and β-anomers of 2-*O*-methyl-D-glucose, and the identification was confirmed by g.l.c.–m.s.

Acid Hydrolysis of the Acetyltetradecamethyl Derivative (7).—A solution of (7) (100 mg) in acetone (5 ml) was added to 10% hydrochloric acid (40 ml). The mixture was refluxed for 2 h, and extracted with chloroform. The chloroform layer was evaporated and the residue was fractionated by preparative t.l.c. (A). Between the two compounds which show yellow spots on t.l.c., one compound (R_F 0.46) was identified as the starting material. The other (R_F 0.05) was isolated as a yellow *syrup* [(11), 13.8 mg] (Found: C, 60.55; H, 4.95. $C_{25}H_{22}N_2O_8\cdot H_2O$ requires C, 60.48; H, 4.87%. Found: M^+, 478.1394. $C_{25}H_{22}N_2O_8$ requires *M*, 478.1376); δ [$(CD_3)_2CO$] 4.04, 3.98, 3.92, 3.62, 3.50 (5 × OMe), 7.47 (s, H-3″), 8.68 (s, H-3′), and 7.9—8.4 (4 H, m, Ar-H).

This product was methylated with diazomethane in ether–methanol, and the main product was identified by g.l.c.–m.s. (column for g.l.c., SE-30; oven temperature, 245 °C) as methyl 4-methoxy-3-(4,5,6-trimethoxy-2-methoxycarbonylphenyl)phenazine-2-carboxylate (12);[4] *m/e* 492 (M^+).

Methanolysis of the Tridecamethyl Derivative (10).—A mixture of (10) (35 mg) and 1% sodium methoxide in methanol (5 ml) was kept at room temperature for 10 min. After neutralization and evaporation, the residue was submitted to preparative t.l.c. [benzene–acetone (10 : 1 v/v)]. A product (R_F 0.60, 8.5 mg, m.p. 131—132 °C, $[\alpha]_D^{23}$ +37°, *c* 1.2, ethanol) was identified as (12) by mixed m.p., ^{1}H n.m.r., and m.s. (M^+, 492).

Deuteriated dimethyl ester (12′) was prepared by treatment of (12) with deuteriomethanol containing sodium. The ^{1}H n.m.r. spectrum [$(CD_3)_2CO$] showed the presence of four methoxy-groups (δ 4.05, 4.00, 3.93, and 3.61); *m/e* 498 (M^+).

Hydrolysis of (12).—A mixture of (12) (100 mg), methanol (1 ml) and 20% potassium hydroxide (3 ml) was heated on a water-bath for 10 min. After acidification, the yellow precipitate was collected and washed with water. Purification by preparative t.l.c. [chloroform–methanol (4 : 1 v/v)] gave (13) as yellow crystals m.p. 147 °C (R_F 0.15, 88.5 mg); δ[$(CD_3)_2CO$] 4.04, 3.99, 3.93, and 3.63 (4 × OMe), 7.54 (s, H-3″), 8.67 (s, H-3′), and 7.9—8.4 (m, 4 H, Ar-H) (Found: M^+, 464.1231. $C_{24}H_{20}N_2O_8$ requires M^+, 464.1220).

Acetylation of (2) *and* (3).—Compound (2) (100 mg) was acetylated with acetic anhydride (2 ml) and pyridine (2 ml) at room temperature. The main product (9) was obtained after preparative t.l.c., system (B), as yellow amorphous *powder* (90 mg) (Found: C, 56.95; H, 3.4; N, 1.55. $C_{73}H_{56}N_2O_{36}$ requires C, 57.04; H, 3.67; N, 1.82%); $\nu_{max.}$ (KBr): 1 780, 1 750 (shoulder), and 1 180 cm^{-1}. The ^{1}H n.m.r. spectrum ($CDCl_3$) showed the presence of thirteen acetyl groups, and the aromatization of C-1′—C-6′ ring by the absence of the methine proton.

Acetylation of (3) gave a product which was identical with (9) by t.l.c., and i.r. and ^{1}H n.m.r. spectra.

(R)-2,2′-*Dimethoxycarbonyl*-4,4′,5,5′,6,6′-*hexamethoxybiphenyl* (15) *from Nona*-O-*methylcorilagin* (14).—Nona-*O*-methylcorilagin (35 mg) was methanolysed in 0.3% sodium methoxide in methanol (1.5 ml) at room temperature. Neutralization (Amberlite IR-120, H^+-form) followed by evaporation and preparative t.l.c. (C) gave dimethyl (+)-hexamethoxydiphenate (15) [(M^+ 450), 11 mg] which was identified with authentic (R)-2,2′-dimethoxycarbonyl-4,4′,5,5′,6,6′-hexamethoxybiphenyl[11] by comparison of u.v. and ^{1}H n.m.r. spectra, and specific rotations.

(R)-2,2′-*Dihydroxymethyl*-4,4′,5,5′,6,6′-*hexamethoxybiphenyl* (16) *from* (15).—Reduction of (15) (16 mg) was carried out with lithium aluminium hydride in dry ether in the same way as that reported by Ikeya *et al.*[11] to give 2,2′-bis(hydroxymethyl)-4,4′,5,5′,6,6′-hexamethoxybiphenyl (16) (9 mg) as colourless needles which were recrystallized from ether–n–hexane, m.p. 106—107 °C ($[\alpha]_D^{15}$ −40.3°, *c* 0.94, $CHCl_3$); *m/e* 394 (M^+), 376 (M^+ −18, 100%), 314, and

302; δ(CCl_4) 6.74 (2 H, s, Ar-H), 3.98 (4 H, s, CH_2OH × 2), 3.86 (6 H, OMe × 2), 3.59 (6 H, OMe × 2), o.r.d. spectra, $[\alpha]_{240}$ +2 800° (*c* 0.034, MeOH).

Measurement of Deuterium-induced Differential Isotope Shift.—The dual cell was composed of two ordinary n.m.r. tubes of 8-mm and 5-mm diameter. The inner tube was supported by the inside wall of the outer tube using two rings made of Teflon. Both the inner tube and the outer tube contained geraniin (250 mg), corilagin (140 mg) or arbutin (120 mg) dissolved in a mixture of $(CD_3)_2CO$ (0.4 ml), and water (0.4 ml, inner tube) or D_2O (0.4 ml, outer tube).

Reduction of Isoterchebin (17).—A solution of isoterchebin (17) (50 mg) and sodium dithionite (75 mg) in methanol–water (4 : 6 v/v, 3 ml) was kept at 70 °C for 10 min. After removing the solvent, the residue was chromatographed on Sephadex LH-20 (2 × 21 cm) column, initially in ethanol and then in methanol. The methanol eluate gave an off-white amorphous powder (16 mg), $[\alpha]_D^{16}$ +58° (*c* 1.0, acetone); δ[$(CD_3)_2CO$] 7.13, 7.01 and 6.98 (2 H each, s), 6.63 and 6.45 (1 H each, s), which was identified with (*S*)-1,2,3-tri-*O*-galloyl-4,6-*O*-hexahydroxydiphenoyl-β-D-glucopyranose by $[\alpha]_D$, i.r., and ^1H n.m.r., and also by h.p.l.c. on a Merck LiChrosorb RP-18 (10 μm) column (4 × 150 mm) in 0.1M H_3PO_4—0.1M KH_2PO_4–ethanol–ethyl acetate (50 : 50 : 2 : 5, by volume).

The same reduction product was also obtained by hydrogenation of isoterchebin over palladium–charcoal followed by Sephadex LH-20 column chromatography.

We thank Dr. H. Taguchi of Tsumura Laboratory for the o.r.d. data, Dr. K. Kawazu, Department of Agricultural Chemistry, Okayama University, for the high-resolution mass spectra, the staff of Hitachi Ltd. and JEOL for measuring some of the ^{13}C n.m.r. spectra, and the staff of Shimadzu Seisakusho, Ltd. for the c.i.–m.s. measurement.

[1/007 *Received*, 5*th January*, 1981]

REFERENCES

[1] Part 11, T. Yoshida and T. Okuda, *Heterocycles*, 1980, **14**, 1473.
[2] T. Okuda, K. Mori, and T. Hatano, *Phytochemistry*, 1980, **19**, 547; T. Okuda, K. Mori, K. Terayama, K. Higuchi, and T. Hatano, *J. Pharm. Soc. Jpn.*, 1979, **99**, 543.
[3] T. Okuda, T. Yoshida, and K. Mori, *J. Pharm. Soc. Jpn.*, 1975, **95**, 1462.
[4] T. Okuda, T. Yoshida, and H. Nayeshiro, *Chem. Pharm. Bull.*, 1977, **25**, 1862.
[5] T. Okuda, H. Nayeshiro, and K. Seno, *Tetrahedron Lett.*, 1977, 4421.
[6] Preliminary report; T. Okuda, T. Yoshida, and T. Hatano, *Tetrahedron Lett.*, 1980, 2561.
[7] Ch. Wünsche, A. Sachs, A. Einwiller, and W. Mayer, *Tetrahedron*, 1968, **24**, 3407.
[8] S. J. Angyal and G. S. Bethell, *Aust. J. Chem.*, 1976, **29**, 1249.
[9] P. E. Pfeffer, K. M. Valentine, and F. W. Parrish, *J. Am. Chem. Soc.*, 1979, **101**, 1265.
[10] K. Mislow, M. A. W. Glass, R. E. O'Brien, P. Rutkin, D. H. Steinberg, J. Weiss, and C. Djerassi, *J. Am. Chem. Soc.*, 1962, **84**, 1455.
[11] Y. Ikeya, H. Taguchi, I. Yosioka, and H. Kobayashi, *Chem. Pharm. Bull.*, 1979, **27**, 1383.
[12] H. Fürstenwerth and H. Schildknecht, *Liebigs Ann. Chem.*, 1976, 112.
[13] T. Okuda, T. Hatano, and T. Yasui, *Heterocycles*, 1981, **16**, 1321.
[14] G. A. Reynold and J. A. VanAllan, *Org. Synth.*, coll. vol. IV, 1963, 15.
[15] C. K. Wilkins and B. A. Bohm, *Phytochemistry*, 1976, **15**, 211.