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Tannins and Related Compounds. VII.[1] Phenylpropanoid-substituted Epicatechins, Cinchonains from *Cinchona succirubra*. (1)

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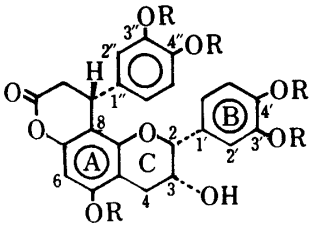
As a result of an investigation of the relatively lower-molecular-weight phenolics in the bark of *Cinchona succirubra* (Rubiaceae), cinchonains Ia (**1**), Ib (**2**), Ic (**3**) and Id (**4**), a new class of flavan-3-ols substituted at the A-ring with a C_6–C_3 unit, have been isolated, together with caffeic acid (**5**) and (−)-epicatechin (**6**). The structures of these compounds have been established on the basis of chemical and spectroscopic evidence. A direct coupling of caffeic acid and (−)-epicatechin leading to the formation of cinchonains has also been achieved.

Keywords——*Cinchona succirubra*; red cinchona; Rubiaceae; cinchonains; phenylpropanoid-substituted flavan-3-ols; caffeic acid; (−)-epicatechin

The bark of the tropical South American genus *Cinchona* (Rubiaceae) is well known as a source of quinine and quinidine, and more than thirty related alkaliods have been isolated. In addition, the presence of a large amount of quinic acid and quinovose[2] in this genus has been demonstrated. However, in contrast to the detailed studies on these compounds, little chemical examination of the phenolic constituents has been made, although the occurrence of polyphenolic compounds designated ambiguously as "cinchona-tannin" has long been recognized owing to its astringency and a strong blue coloration of the extract with the ferric chloride reagent. As part of a chemical study on tannins and related compounds in crude drugs, we have investigated the polyphenolic constituents of red cinchona, the bark of *Cinchona succirubra* PAVON *et* KLOTZSCH, and isolated four new phenylpropanoid-substituted flavan-3-ols, named cinchonains Ia—Id (**1**—**4**), together with caffeic acid (**5**) and (−)-epicatechin (**6**). This paper deals with the isolation and structure determination of these compounds.

The commercial red cinchona was extracted with 80% aqueous acetone and the extracts were treated as shown in Chart 1 to give cinchonains Ia—Id (**1**—**4**), caffeic acid (**5**) and (−)-epicatechin (**6**). The structures of the latter two compounds were confirmed by comparisons of their physical and spectral data with those of authentic samples. In addition, the absence of catechin, which usually coexists with epicatechin in plant tissues, was confirmed by high-performance liquid chromatographic (HPLC) analysis using a reverse-phase (ODS) column.

Cinchonain Ia (**1**), colorless needles (H_2O), mp 173—175°C, $[\alpha]_D$ −214° (acetone), $C_{24}H_{20}O_9 \cdot H_2O$, exhibits a molecular ion peak at m/z 452 in the field-desorption mass spectrum (FD-MS). The occurrence of a flavan-3-ol skeleton in the molecule could be easily deduced from the proton nuclear magnetic resonance (^{1}H-NMR) spectrum. The signals at δ 4.89 (s), 4.30 (m) and 2.90 (2H, m) ascribable to C_2–, C_3– and C_4–H on a flavan C-ring, respectively, and ABX-type aromatic signals at δ 6.72 (dd, J=2, 8 Hz), 6.84 (d, J=8 Hz) and 7.08 (d, J=2 Hz) arising from the B-ring protons are closely related to those in epicatechin (Table I). A singlet signal at high field in the aromatic region (δ 6.24) suggests that the A-ring is tri-substituted. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum (Table II) reveals, in addition to fifteen signals similar to those of epicatechin, the presence of a methine (δ 34.5, d), a methylene (δ 38.0, t) and an aromatic ring with a 3,4-dihydroxy substitution system [δ 114.4 (2C), d; 118.4, d; 134.5, s; 144.1, s; 144.7, s]. Furthermore, the appearance of a signal at δ 168.9 suggests the occurrence of a carboxyl function. This is supported by the infrared



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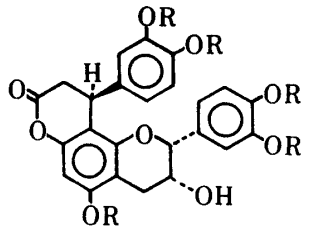

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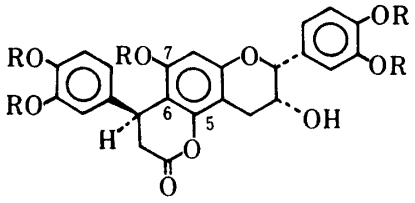

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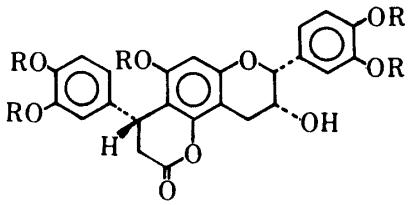

4 : R=H
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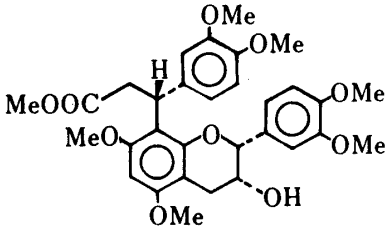
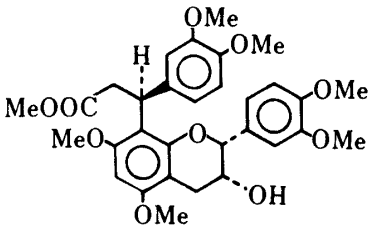
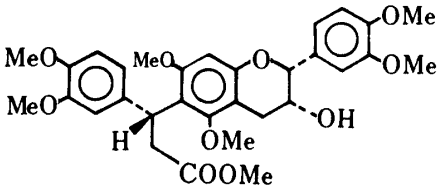


1b



2b



4b

(IR) spectrum showing a strong band at 1750 cm^{-1}, probably due to a γ,δ-unsaturated six-membered lactone.

Mehtylation of **1** with dimethyl sulfate and potassium carbonate in dry acetone afforded a methyl ether (**1a**), which exhibits signals due to five methoxyl groups [δ3.56, 3.76, 3.80, 3.88 ($\times$ 2)] in the ^{1}H-NMR spectrum. Compound **1a** was further treated with dimethylsulfate and potassium carbonate in a mixture of acetone and methanol (1 : 1) to furnish a heptamethylate (**1b**). This indicates that opening of the lactone ring in **1a** and subsequent methylation of the resulting carboxylic acid and phenolic hydroxyl group took place in this alkaline medium. No methylation was achieved at the C_3-alcoholic hydroxyl group as revealed by the elemental analysis and the molecular ion at *m/z* 568 in the electron-impact mass spectrum (EI-MS). Careful examination of fragment peaks in the EI-MS of **1b** provided information consistent with that anticipated (Chart 2). A prominent peak at *m/z* 388 is easily assignable to the fragment (a) formed by a reverse Diels–Alder fission characteristic of flavonoids. Loss of 73 mass units (CH_2COOCH_3) from this ion and from the molecular ion gives rise to peaks at *m/z* 315 (b) and 495 (c), respectively, providing evidence of a six-membered lactone ring in **1**. The

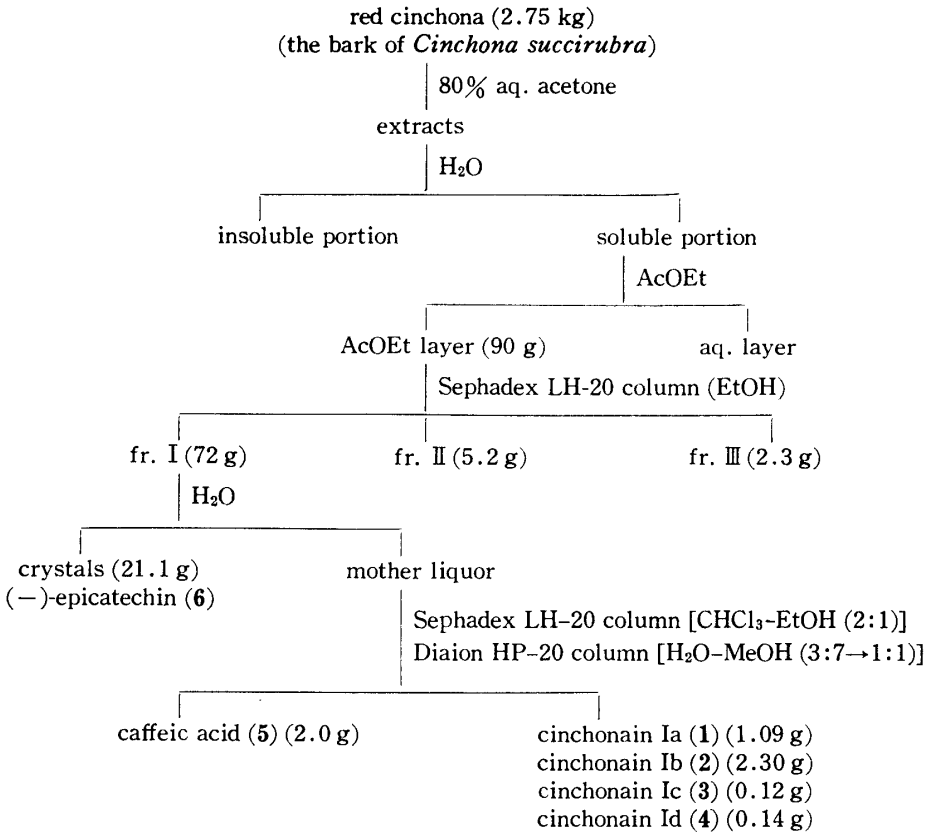


Chart 1

TABLE I. ^{1}H-NMR Spectra of Cinchonains (**1**, **2**, **3** and **4**) and Epicatechins (**6**)[a]

	1	2	3	4	6
C_2–H	4.89(s)	4.99(s)	4.93(s)	4.93(s)	4.88(s)
C_3–H	4.30(m)	4.26(m)	4.26(m)	4.27(m)	4.20(m)
C_4–H	2.90(m)	2.90(m)	2.92(m)	2.92(m)	2.80(m)
C_6–H	6.24(s)	6.24(s)			6.12(d, J=2)
C_8–H			6.29(s)	6.29(s)	6.22(d, J=2)
$C_{2'}$–H	7.08(d, J=2)	6.93(d, J=2)	7.08(br s)	7.07(br s)	7.04(d, J=2)
$C_{5'}$–H	6.84(d, J=8)	6.72(d, J=8)	6.88(d, J=8)	6.88(d, J=8)	6.86(d, J=8)
$C_{6'}$–H	6.72(dd, J=2,8)	6.64(dd, J=2,8)	6.78(d, J=8)	6.78(d, J=8)	6.78(dd, J=2, 8)
$C_{2''}$–H	6.59(d, J=2)	6.68(d, J=2)	6.64(d, J=2)	6.64(d, J=2)	
$C_{5''}$–H	6.46(dd, J=2,8)	6.58(dd, J=2,8)	6.52(dd, J=2,8)	6.50(dd, J=2,8)	
$C_{6''}$–H	6.64(d, J=8)	6.74(d, J=8)	6.62(d, J=8)	6.62(d, J=8)	
α-H	2.85(dd, J=2,16)	2.72—3.18(m)	2.7—3.2(m)	2.7—3.2(m)	
	3.12(dd, J=6,16)				
β-H	4.54(dd, J=2,6)	4.47(dd, J=2,6)	4.47(dd, J=2,6)	4.46(dd, J=2,6)	

a) Spectra were run in acetone-d_6 at 100 MHz. s, singlet; d, doublet; m, multiplet. J-values are expressed in Hz.

most significant feature in the EI-MS is the appearance of the base peak at m/z 223 derived from a radical ion of dimethyl dihydrocaffeic acid methyl ester (d). This observation clearly indicates the presence of a C_6–C_3 (phenylpropanoid) unit which is linked to the epicatechin A-ring when considered in conjunction with the above ^{1}H- and ^{13}C-NMR evidence.

The location of the C_6–C_3 unit was deduced from comparisons of the ^{13}C-NMR resonances arising from A-ring in **1a** with those in methyl derivatives of gambiriins A_1 (**7**) and A_3 (**8**) isolated previously from gambir.[3] Careful examination of the ^{13}C-NMR spectra of the methyl

Table II. ^{13}C-NMR Spectra of Cinchonains (**1**, **2**, **3** and **4**) and Epicatechin (**6**)[d]

	1	2	3	4	6
C_2	79.0(d)	79.4(d)	79.2(d)	79.2(d)	79.3(d)
C_3	65.8(d)	66.0(d)	65.7(d)	66.0(d)	66.9(d)
C_4	28.9(t)	28.8(t)	28.3(t)	28.3(t)	28.9(t)
C_5	156.2(s)	156.2(s)	153.5(s)	153.5(s)	157.5(s)
C_6	95.8(d)	96.0(d)	106.2(s)	106.3(s)	95.7(d)
C_7	151.2(s)	151.2(s)	155.1(s)	155.3(s)	157.5(s)
C_8	105.2(s)	105.4(s)	99.4(d)	99.4(d)	96.2(d)
C_{4a}	104.5(s)	104.7(s)	100.3(s)	100.4(s)	99.7(s)
C_{8a}	152.5(s)	152.6(s)	151.5(s)	151.5(s)	157.0(s)
$C_{1'}$	131.2(s)	131.0(s)	131.0(s)	131.2(s)	132.2(s)
$C_{2'}$	114.4(d)[a]	114.5(d)[a]	114.7(d)[a]	114.7(d)[a]	115.2(d)[a]
$C_{3'}$	144.1(s)[b]	144.3(s)[b]	144.0(s)[b]	144.1(s)[b]	145.1(s)[b]
$C_{4'}$	144.7(s)[b]	144.8(s)[b]	144.7(s)[b]	144.9(s)[b]	145.3(s)[b]
$C_{5'}$	114.4(d)[a]	114.8(d)[a]	114.7(d)[a]	114.8(d)[a]	115.5(d)[a]
$C_{6'}$	118.4(d)	118.7(d)[c]	118.4(d)[c]	118.9(d)[c]	119.3(d)
$C_{1''}$	134.5(s)	134.4(s)	134.1(s)	134.3(s)	
$C_{2''}$	115.4(d)[a]	115.3(d)[a]	115.4(d)[a]	115.4(d)[a]	
$C_{3''}$	145.0(s)[b]	144.9(s)[b]	144.7(s)[b]	144.9(s)[b]	
$C_{4''}$	145.4(s)[b]	145.5(s)[b]	145.3(s)[b]	145.5(s)[b]	
$C_{5''}$	115.8(d)[a]	115.9(d)[a]	115.9(d)[a]	115.8(d)[a]	
$C_{6''}$	118.4(d)	118.5(d)[c]	118.8(d)[c]	118.9(d)[c]	
α-C	38.0(t)	37.6(t)	37.7(t)	37.7(t)	
β-C	34.5(d)	34.2(d)	34.5(d)	34.4(d)	
–COO–	168.9(s)	168.9(s)	169.2(s)	168.8(s)	

Assignments with the superscript *a*), *b*) or *c*) may be interchanged in each column.
d) Spectra were run in acetone-d_6+D_2O at 25.05 MHz. s, singlet; d, doublet; t, triplet.

ethers (**7a** and **8a**, respectively) revealed that there are highly diagnostic differences in C_6-, C_8- and C_{4a}-chemical shifts which make it possible to distinguish between the C_8- and C_6-substitution systems. In **7a**, an 8-substituted catechin, all these signals were observed at higher field [C_6: δ 88.6 (d); C_{4a}: 102.5 (s); C_8: 112.2 (s)] than those in the alternative C_6-substituted catechin (**8a**) [C_8: δ 96.1 (d); C_{4a}: 108.4 (s); C_6: 117.5 (s)]. Compound **1a** exhibits signals for these carbons at δ 89.6 (d), 100.6 (s) and 111.3 (s), and these values are in agreement with ose found in the above C_8-substituted catechin, thus indicating the occurrence of a carbon-carbon linkage at the C_8-position in the epicatechin moiety. As regards the stereostructure of **1**, a comparative study on the ^{1}H–NMR and circular dichroism (CD) spectra of **1** and cinchonain Ib (**2**) has been made, and the results are discussed later in this paper.

Cinchonain Ib (**2**), colorless needles (H_2O), mp 245—247°C, $[\alpha]_D$ +12° (acetone), $C_{24}H_{20}O_9$, *m/z* 452 (M^+, FD-MS), contains in the molecule a flavan-3-ol framework with epicatechin stereochemistry (C_2, C_3: *cis*) and a 3,4-dihydroxyphenylpropanoid substituent as indicated by the ^{1}H- and ^{13}C-NMR spectra (Table I and II) analogous to those of **1**. Methylation with dimethyl sulfate and potassium carbonate in dry acetone furnished the pentamethyl ether (**2a**). This was further methylated under the same conditions as for **1a** to yield the heptamethylate (**2b**). The EI-MS of **2b** exhibits a molecular ion at *m/z* 568 and fragment peaks at *m/z* 495, 388, 315, 223 and 181 identical to those found in **1b** (Chart 2). The ^{13}C-NMR spectrum of **2b** is also similar to that of **1b**, showing signals due to C_6, C_{4a} and C_8 at δ 89.0 (d), 100.7 (s) and 111.5 (s), respectively, and this indicates that the phenylpropanoid unit is located at the C_8-position of the epicatechin moiety. Accordingly, **2** should be a configurational isomer of **1**.

The stereostructures of **1** and **2** were deduced by analysis of their ^{1}H-NMR spectra. In the spectrum of **1**, signals due to the C_2–H and the ABX-type B-ring protons are observed at positions in accordance with those of epicatechin . On the other hand, **2** displays the C_2-H

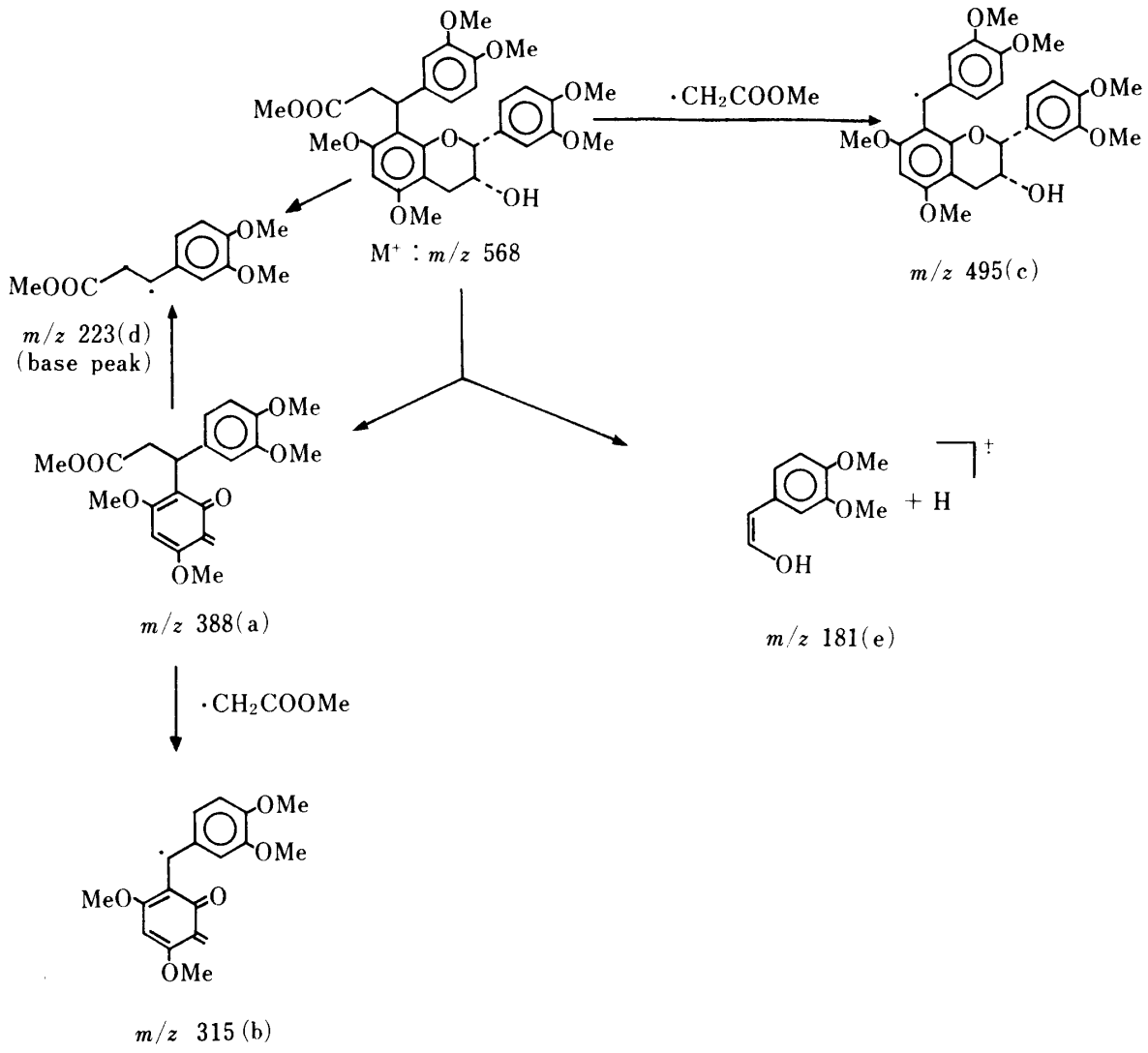
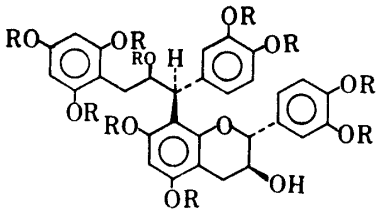


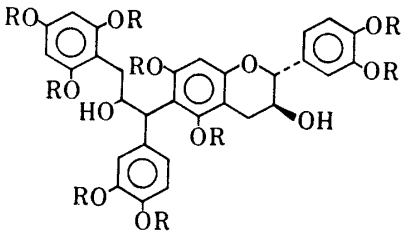
Chart 2

signal at δ 4.99 and the B-ring proton signals at δ 6.64 (dd, J=2, 8 Hz), 6.72 (d, J=8 Hz) and 6.93 (d, J=2 Hz), the C_2–H signal being shifted downfield while the other three signals are shifted upfield as compared with those of epicatechin (Table I), This implies that these protons are anisotropically affected by the aromatic ring in the phenylpropanoid moiety. Since **1** and **2** give the same coupling constants for the benzylic methine protons (J=2, 6 Hz), the dihedral angles between this proton and the neighboring methylene protons are presumed to be similar, suggesting a conformational difference of the lactone ring in **1** and **2** , as illustrated in Fig. 1. The downfield shift of the C_2–H signal in **2** may be explained if the aromatic ring in the phenylpropanoid moiety and the C_2–H are located on the same side of the plane. This was further supported by the observation that the C_2–H signal was shifted abnormally downfield (δ 5.66) in cinchonain IIb,[4] which contains an additional epicatechin moiety at the C_4-position of **2**. In contrast, such abnormality was not observed in the ^{1}H-NMR spectrum of cinchonain IIa[4] which has cinchonain Ia moiety in the molecule. On the basis of these findings, the relative stereostructures of cinchonains Ia and Ib are presumed to be **1** and **2**, respectively.

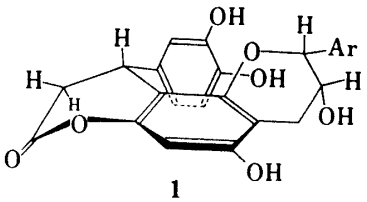
It should be noted that the presence of a non-bonded interaction in **2** between the aromatic ring in the C_6–C_3 unit and the C_2–H (along with the B-ring), as can be seen from the ^{1}H-NMR



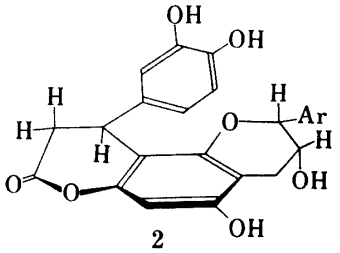
7 : R=H
7a : R=Me



8 : R=H
8a : R=Me



1



2

Fig. 1

signals, provides further support for the location on the substituent at the C_8-position, since in the case of a C_6-substitution system, such an intramolecular interaction would not be expected.

Cinchonain Ic (**3**), colorless needles (H_2O), mp >300°C, $[\alpha]_D$ −25.1° (acetone), and cinchonain Id (**4**), colorless needles (H_2O), mp 182—183°C, $[\alpha]_D$ +29.1° (acetone), $C_{24}H_{20}O_9 \cdot H_2O$, give closely related ^{1}H- and ^{13}C-NMR spectra which are virtually indistinguishable (Tables I and II). Their ^{1}H-NMR spectra are almost identical with that of **1**, indicating that the same functional groups are present in each case, and that there is no intramolecular interaction between the C_2–H (along with the B-ring) and the substituents. The significant structural differences between **1** and these compounds could be deduced from the ^{13}H-NMR signals arising from the A-ring, especially from those of C_6, C_8 and C_{4a}, although the other signals are closely related. A signal at δ 99.4 appearing as a doublet in the off-resonance spectrum is assigned to C_8 which resonates at lower field than C_6 in **1** and **2**, in agreement with the chemical shift differences between C_6 and C_8 commonly observed in the ^{13}C-NMR spectra of flavonoids with a 5,7-substituted A-ring. The assignment of a singlet signal at δ 106.2 in **3** (δ 106.3 in **4**) to C_6 was based on a comparison of the chemical shift with those of procyanidins reported previously.[5] The remaining singlet at δ 100.3 in **3** (δ 100.4 in **4**) is therefore assignable to C_{4a}. This signal pattern of the A-ring suggests that the substituent is at the C_6-position. Compounds **3** and **4** yielded, on methylation with dimethyl sulfate and potassium carbonate in dry acetone, pentamethylates (**3a** and **4a**, respectively) whose ^{1}H-NMR spectra are almost identical. Further methylation of **4a** in the same way as descrided above afforded the heptamethylate (**4b**). The ^{13}C-NMR spectrum of **4a** exhibits signals due to C_8, C_{4a} and C_6 at δ 96.4, 104.8 and 118.2, respectively, in close agreement with those observed in the nonamethylate of gambiriin A_3 (**8**), a C_6-substituted catechin (C_8: δ 96.1; C_{4a}: 106.3; C_6: 117.5), thus confirming that the phenylpropanoid moiety is linked through a carbon-carbon bond to the C_6-position.

Next, the problem of whether the lactone ring closes at the C_5- or C_7-hydroxyl group could be solved by nuclear Overhauser effect measurement in **4a**. Irradiation at the methoxyl region (δ 3.88)[6] resulted in a 16% enhancement of the integrated peak intensity of C_8–H at δ 6.40 . This clearly indicates that a methoxyl group is present at the C_7-position, and that the lactone ring is therefore formed through the C_5-hydroxyl.

The relative configuration of a methine carbon β to the carboxyl group in **3** and **4** was

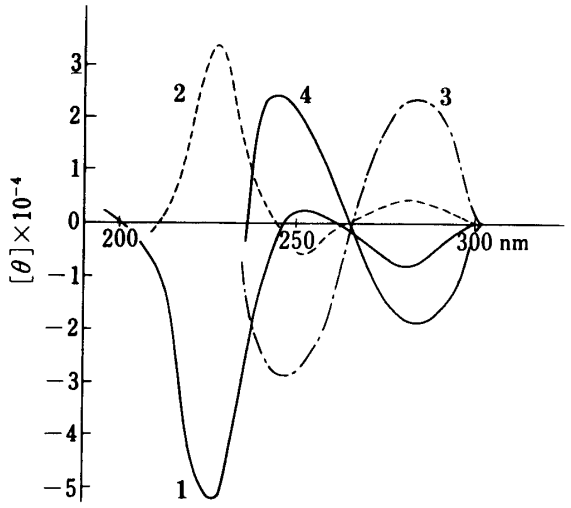


Fig. 2. CD Spectra of Cinchonains (1, 2, 3 and 4)

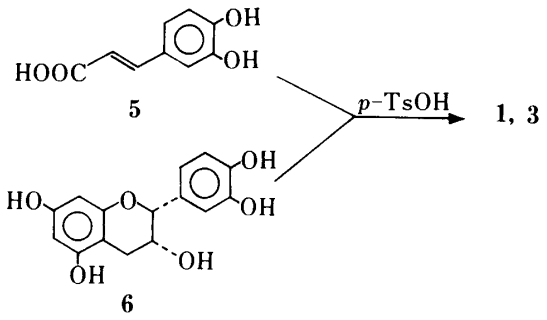


Chart 3

established by comparisons of their circular dichroism (CD) spectra with those of **1** and **2**. As shown in Fig. 1, **1** and **2** show CD bands of opposite signs, indicating that these bands are mainly due to chirality of the β-carbon, being largely unaffected by the asymmetrical C_2 and C_3. Similarly, **3** and **4** exhibit curves of opposite signs analogous to those of **2** and **1**, respectively. From these observations, the configurations at the β-carbon in **3** and **4** were concluded to correspond to those in **2** and **1**, respectively.

In order to establish the absolute stereostructures of cinchonains and also to establish their structures definitively, we attempted to prepare these compounds by coupling of caffeic acid and (−)-epicatechin, the latter being of known absolute configurations at chiral centers C_2 (*R*) and C_3 (*R*). Application of reported condensation methods often used in the flavonoid field (BF_3-etherate,[7] dilute hydrochloric acid[8]) to the coupling of these compounds was unsuccessful. However, treatment of the mixture in dry dioxane in the presence of *p*-toluenesulfonic acid resulted in the formation of at least six products, of which two were separable by chromatography over high-porous polystyrene gel and were identified as cinchonains Ia (**1**) and Ic (**3**) by comparisons of the mp's, $[\alpha]_D$ and ^{1}H-NMR spectra.

Consequently, the structures of cinchonains Ia, Ib, Ic and Id, including the absolute configurations, are established as **1**, **2**, **3** and **4**, respectively.

Cinchonains have also been isolated from *Uncaria rhynchophylla* Miquel (Rubiaceae), *Kandelia candel* L. (Rhizophoraceae), *Polygonum bistorta* L. (Polygonaceae), and *Raphiolepis umbellata* Makino (Rosaceae), and this raises the possibility of these compounds being quite widely distributed in the plant kingdom.

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-4 digital polarimeter (cell length: 0.5 dm). IR spectra were recorded with a JASCO IR-G spectrometer, FD-MS and EI-MS with JEOL D 300 and JEOL DX 300 spectrometers. ^{1}H- and ^{13}C-NMR spectra were taken with JEOL PS-100 and JEOL FX-100 spectrometers, respectively, with tetramethylsilane as an internal standard, and chemical shifts are given on a δ (ppm) scale. CD data were obtained with a JASCO J-20 spectropolarimeter in methanol. Column chromatography was carried out with Sephadex LH-20 (25—100 μ, Pharmacia Fine Chemical Co. Ltd.), Diaion HP-20 AG (75—150 μ, Mitsubishi Chemical Industries Ltd.) and Kieselgel 60 (70—230 mesh, Merck). Thin-layer chromatography (TLC) was performed on precoated Kieselgel 60 F_{254} plates (0.20 mm, Merck) with (A) benzene–ethanol (4: 1) and (B) benzene–acetone–acetic acid (5: 4: 1) as solvent systems, and the spots were detected by the use of anisaldehyde–sulfuric acid and ferric chloride reagent sprays. HPLC was conducted

on a Toyo Soda apparatus equipped with an SP 8700 solvent delivery system and a UV-8 model II spectrophotometer using an LS 410 column (5 μ, 4 mm i.d. × 300 mm) [solvent, CH_3CN–H_2O (3: 7): flow rate, 0.75 ml/min].

Extraction and Isolation——Commercial red cinchona (the bark of *Cinchona succirubra*) (2.75 kg) was finely powdered and extracted by percolation with 80% aqueous acetone. The acetone was removed by evaporation under reduced pressure. The aqueous solution thus obtained was allowed to stand at room temperature to afford brown precipitates, which were negative to the ferric chloride reagent and were removed by filtration. The filtrate (*ca.* 6 l) was extracted eight times with AcOEt (*ca.* 600 ml each). The combined AcOEt layer was concentrated to afford a brown powder (90 g), which was subjected to column chromatography on Sephadex LH-20 using EtOH as an eluent to yield fractions; fr. I (72 g), II (5.2 g) and III (2.3 g). Fr. I was crystallized from H_2O to give colorless needles, mp 243—244°C, $[\alpha]_D^{28}$ −54.0° (c=0.5, acetone); this product was identified as (−)-epicatechin (**6**) by direct comparison of the IR and ^{1}H-NMR spectra with those of an authentic sample. Chromatography of the mother liquor on Sephadex LH-20 with $CHCl_3$–EtOH (2: 1) furnished caffeic acid (**5**), a yellow powder, mp 223—225°C. Further elution of this column with the same solvent system afforded a mixture which was subsequently chromatographed on Diaion HP-20 AG. Elution with an increasing amount of MeOH in H_2O (3: 7—1: 1) followed by crystallization of each fraction from H_2O gave cinchonains Ia (**1**) (1.09 g), Ib (**2**) (2.3 g), Ic (**3**) (0.12 g) and Id (**4**) (0.14 g).

Cinchonain Ia (**1**)——Colorless needles (H_2O), mp 173—175°C, $[\alpha]_D^{32}$ −214° (c=0.97, acetone). *Anal.* Calcd for $C_{24}H_{20}O_9 \cdot H_2O$: C, 61.27; H, 4.72. Found: C, 61.02; H, 4.65. FD-MS m/z: 452 (M^+). IR ν_{max}^{KBr} cm^{-1}: 3360—3460 (OH), 1750 (γ,δ-unsatd. six-membered lactone), 1620 (aromatic ring). ^{1}H-NMR: Table I. ^{13}C-NMR: Table II. CD: Fig. 1.

Hexaacetate of 1——**1** (50 mg) was acetylated with acetic anhydride (1.0 ml) and dry pyridine (0.5 ml) overnight at room temperature. Excess reagents were removed by blowing N_2 gas over the solution, and the residue was purified by silica gel column chromatography with benzene–acetone (19: 1) to furnish the hexaacetate as a white powder (52 mg), $[\alpha]_D^{27}$ +28.7° (c=1.0, acetone). *Anal.* Calcd for $C_{36}H_{32}O_{15}$: C, 61.36; H, 4.58. Found: C, 61.32; H, 4.78. ^{1}H-NMR ($CDCl_3$): 1.92, 2.16, 2.21, 2.30 (×3) (each 3H, s, OAc), 2.7—3.3 (4H, m, C_4–H, α-H), 4.62 (1H, dd, J=4, 6 Hz, β-H), 5.08 (1H, s, C_2–H), 5.40 (1H, t-like, C_3–H), 6.58 (1H, s, C_6–H), 6.92—7.32 (6H, m, aromatic H).

Pentamethylate (Ia) of 1——A mixture of **1** (100 mg), anhydrous potassium carbonate (1.0 g) and dimethyl sulfate (0.7 ml) in dry acetone (8 ml) was refluxed for 4 h with stirring. After removal of inorganic salts, the filtrate was concentrated to a syrup, which was chromatographed over silica gel using benzene–acetone (9: 1) to yield the pentamethyl ether (**1a**) as a white amorphous powder (126 mg), $[\alpha]_D^{27}$ −130.5° (c=0.99, acetone). *Anal.* Calcd for $C_{29}H_{30}O_9 \cdot 1/2H_2O$: C, 65.52; H, 5.88. Found: C, 65.81; H, 5.92. EI-MS m/z: 522 (M^+). ^{1}H-NMR ($CDCl_3$): 2.9—3.1 (4H, m, C_4–H, α-H), 3.56, 3.76, 3.80, 3.88 (×2) (each 3H, s, OMe), 4.32 (1H, m, C_3–H), 4.59 (1H, t, J=4 Hz, β-H), 4.86 (1H, s, C_2–H), 6.32 (1H, s, C_6–H), 6.56—7.10 (6H, m, aromatic H).

Heptamethylate (Ib) of 1——A mixture of **1a** (34 mg), anhydrous potassium carbonate (0.5 g) and dimethyl sulfate (0.3 ml) in MeOH–acetone (1: 1) (10 ml) was refluxed for 30 min. Filtration and concentration of the filtrate under reduced pressure gave an oil which was purified by silica gel chromatography with benzene-acetone (9: 1) to furnish the heptamethylate (**1b**) as a white amorphous powder (40 mg), $[\alpha]_D^{27}$ −40.1° (c=1.0, acetone). *Anal.* Calcd for $C_{31}H_{36}O_{10}$: C, 65.48; H, 6.38. Found: C, 65.49; H, 6.48. EI-MS m/z (%): 568 (M^+, 42), 495 (fragment c, 22), 388 (a, 56), 315 (b, 64), 223 (d, 100), 181 (e, 36), 167 (41), 151 (52). ^{1}H-NMR ($CDCl_3$): 2.92 (2H, m, C_4–H), 3.22 (1H, d, J=8 Hz, α-H), 3.52, 3.68, 3.78 (×4), 3.86 (each 3H, s, OMe), 4.24 (1H, m, C_3–H), 4.96 (1H, s, C_2–H), 5.21 (1H, t, J=8 Hz, β-H), 6.14 (1H, s, C_6–H), 6.64—7.06 (6H, m, aromatic H). ^{13}C-NMR ($CDCl_3$): 28.4 (t, C_4), 35.6 (d, β-C), 38.4 (t, α-C), 51.3, 55.2, 55.5, 55.7 (×2), 55.8, 56.2 (OMe), 66.1 (d, C_3), 78.2 (d, C_2), 89.0 (d, C_6), 100.6 (s, C_{4a}), 109.4, 110.7, 111.0 (×2) (each d, $C_{2',2'',5',5''}$), 111.3 (s, C_8), 118.0, 119.2 (each d, $C_{6',6''}$), 130.7 (s, $C_{1'}$), 136.6 (s, $C_{1''}$), 146.7, 148.1, 148.3, 148.8 (each s, $C_{3',3'',4',4''}$), 15.26 (s, C_{8a}), 156.9, 157.2 (each s, $C_{5,7}$), 173.1 (s, -COO-).

Cinchonain Ib (**2**)——Colorless needles (H_2O), mp 245—247°C, $[\alpha]_D^{32}$ +12.1° (c=0.41, acetone). *Anal.* Calcd for $C_{24}H_{20}O_9$: C, 63.71; H, 4.46. Found: C, 63.56; H, 4.40. FD-MS m/z: 452 (M^+). IR ν_{max}^{KBr} cm^{-1}: 3480 (br, OH), 1745 (γ,δ-unsatd. six-membered lactone), 1610 (aromatic ring). ^{1}H-NMR: Table I. ^{13}C-NMR: Table II. CD: Fig. 1.

Hexaacetate of 2——**2** (50 mg) was acetylated with acetic anhydride (1.0 ml) and dry pyridine (0.5 ml). Work-up as before yielded the hexaacetate as a white powder (37 mg), $[\alpha]_D^{27}$ +42.7° (c=1.0, acetone). *Anal.* Calcd for $C_{36}H_{32}O_{15}$: C, 61.36; H, 4.58. Found: C, 61.17; H, 4.86. ^{1}H-NMR ($CDCl_3$): 1.80, 2.16, 2.23, 2.26, 2.28, 2.32 (each 3H, OAc), 2.92 (2H, d-like, C_4–H), 3.05 (2H, d-like, J=4 Hz, α-H), 4.60 (1H, t, J=4 Hz, β-H), 5.12 (1H, s, C_2–H), 5.31 (1H, m, C_3–H), 6.60 (1H, s, C_6–H), 6.9—7.2 (6H, aromatic H).

Pentamethylate (2a) of 2——A mixture of **2** (205 mg), anhydrous potassium carbonate (2.0 g) and dimethyl sulfate (1.5 ml) in dry acetone (15 ml) was refluxed for 6 h with stirring. The mixture was treated as described for **1** to give the pentamethylate (**2a**) as a white amorphous powder (244 mg). *Anal.* Calcd for $C_{29}H_{30}O_9 \cdot 1/2H_2O$: C, 65.52; H, 5.88. Found: C, 65.97; H, 6.01. EI-MS m/z: 522 (M^+). ^{1}H-NMR ($CDCl_3$): 2.76—3.16 (4H, m, C_4–H, α-H), 3.57, 3.74, 3.80, 3.82, 3.83 (each 3H, s, OMe), 4.22 (1H, m, C_3–H), 4.56 (1H, t-like, J=4 Hz, β-H), 5.03 (1H, s, C_2–H), 6.33 (1H, s, C_6–H), 6.6—6.8 (6H, m, aromatic H).

Heptamethylate (2b) of 2——A mixture of **2b** (34 mg), anhydrous potassium carbonate (0.6 g) and dimethyl sulfate (0.4 ml) was refluxed for 40 min. The reaction mixture was worked up as before to yield the heptamethylate (**2b**) as a white amorphous powder (37 mg). *Anal.* Calcd for $C_{31}H_{36}O_{10}$: C, 65.48; H, 6.38; Found: C, 65.17; H, 6.54. EI-MS: almost identical to that of **1b**. ^{1}H-NMR ($CDCl_3$): 2.88 (2H, m, C_4–H), 3.21 (1H, dd, J=8, 14 Hz, α-H), 3.40 (1H, dd, J=8, 14 Hz, α-H), 4.20 (1H, m, C_3–H), 4.84 (1H, s, C_2–H), 5.16 (1H, t, J=8 Hz, β-H), 6.14 (1H, s, C_6–H), 6.6—7.2 (6H, m, aromatic H). ^{13}C-NMR ($CDCl_3$): 28.8 (t, C_4), 35.8 (d, β-C), 38.1 (t, α-C), 51.3, 55.2 (×2), 55.7 (×2), 55.9, 56.1 (each q, OMe), 65.9 (d, C_3), 78.6 (d, C_2), 89.0 (d, C_6), 100.7 (s, C_{4a}), 109.8, 110.6, 110.7, 110.9 (each d, $C_{2',2'',5',5''}$), 111.5 (s, C_8), 130.8 (s, $C_{1'}$), 136.3 (s, $C_{1''}$), 146.7, 148.0, 148.5, 148.8 (each s, $C_{3',3'',4',4''}$), 15.29 (s, C_{8a}), 156.5, 157.2 (each s, $C_{5,7}$), 173.1 (–COO–).

Cinchonain Ic (3)——Colorless needles (H_2O), mp >300°C, $[\alpha]_D^{24}$ −25.1° (c=0.22, acetone). IR ν_{max}^{KBr} cm^{-1}: 3300 (br, OH), 1740 (γ,δ-unsatd. six-membered lactone). ^{1}H-NMR: Table I. ^{13}C-NMR: Table II. CD: Fig. 1.

Pentamethylate (3a) of 3——**3** (47 mg) in dry acetone (6 ml) was methylated for 2.5 h with anhydrous potassium carbonate (0.8 g) and dimethyl sulfate (0.5 ml). The mixture was worked-up as before, yielding the heptamethylate (**3a**) as a white amorphous powder, $[\alpha]_D^{27}$ −36.9° (c=1.0, acetone). ^{1}H-NMR ($CDCl_3$): 2.95—3.16 (4H, m, C_4–H, α-H), 3.75, 3.79, 3.82, 3.89, 3.91 (each 3H, s, OMe), 4.31 (1H, m, C_3–H), 4.51 (1H, dd, J=4, 6 Hz, β-H), 4.99 (1H, s, C_2–H), 6.41 (1H, s, C_8–H), 6.53—7.10 (6H, m, aromatic H).

Cinchonain Id (4)——Colorless needles (H_2O), mp 182—183°C, $[\alpha]_D^{24}$ +29.1° (c=0.33, acetone). *Anal.* Calcd for $C_{24}H_{20}O_9 \cdot H_2O$: 61.27; H, 4.72. Found: C, 60.75; H, 4.73. IR ν_{max}^{KBr} cm^{-1}: 3300—3460 (br, OH), 1740—1750 (γ,δ-unsatd. six-membered lactone). ^{1}H-NMR: Table I. ^{13}C-NMR: Table II. CD: Fig. 1.

Pentamethylate (4a) of 4——A mixture of **4** (92 mg), anhydrous potassium carbonate (1.0 g) and dimethyl sulfate (0.7 ml) in dry acetone (8 ml) was refluxed for 6 h. Treatment of the reaction mixture as before, followed by crystallization from EtOH afforded the pentamethylate (**4a**) as colorless needles, mp 238—239°C, $[\alpha]_D^{27}$ +9.9° (c=0.99, acetone–$CHCl_3$). ^{1}H-NMR ($CDCl_3$): 2.94—3.10 (4H, m, C_4–H, α-H), 3.76, 3.80, 3.81, 3.88, 3.91 (each 3H, s, OMe), 4.31 (1H, m, C_3–H), 4.51 (1H, dd, J=4, 6 Hz, β-H), 5.01 (1H, s, C_2–H), 6.40 (1H, s, C_8–H), 6.57—7.10 (6H, m, aromatic H).

Heptamethylate (4b) of 4——**4a** (28 mg) was treated as described for **1a** to give the heptamethylate (**4b**) as a white amorphous powder (32 mg), $[\alpha]_D^{27}$ −62.0° (c=0.99, acetone). ^{1}H-NMR ($CDCl_3$): 3.01 (2H, m, C_4–H), 3.10 (1H, dd, J=8, 14 Hz, α-H), 3.32 (1H, dd, J=8, 14 Hz, α-H), 4.24 (1H, m, C_3–H), 4.96 (1H, s, C_2–H), 5.02 (1H, t, J=8 Hz, β-H), 6.37 (1H, s, C_8–H), 6.66—7.08 (6H, m, aromatic H). ^{13}C-NMR ($CDCl_3$): 28.8 (t, C_4), 36.4 (d, β-C), 38.1 (t, α-C), 51.4, 55.4, 55.7 (×2), 60.5 (each q, OMe), 78.2 (d, C_2), 96.4 (d, C_8), 104.8 (s, C_{4a}), 109.5, 110.6, 111.0, 111.1 (each d, $C_{2',2'',5',5''}$), 118.2 (s, C_6), 118.4, 119.4 (each d, $C_{6',6''}$), 130.5 (s, $C_{1'}$), 136.3 (s, $C_{1''}$), 146.8, 148.1, 148.6, 148.8 (each s, $C_{3',3'',4',4''}$), 153.7, 157.4, 157.7 (each s, $C_{5,7,8a}$), 173.1 (s, –COO–).

Preparation of Cinchonains——A mixture of (−)-epicatechin (1.1 g), caffeic acid (1.0 g) and *p*-toluenesulfonic acid (0.3 g) in dry dioxane was heated for 3 h on a boiling water bath. The reaction mixture, after removal of the solvent by evaporation, was applied to a Diaion HP-20 column. Elution with MeOH–H_2O (45: 55) afforded colorless needles (H_2O) (70 mg), mp 172—174°C, $[\alpha]_D^{20}$ −224° (c=1.45, acetone), the ^{1}H-NMR spectrum of which was superimposable on that of cinchonain Ia (**1**). Further elution with the same solvent system yielded colorless needles (H_2O) (1.4 mg), mp 182—183°C, $[\alpha]_D^{20}$ +27.1° (c=0.14, acetone), which were identified as conchonain Id (**4**) by direct comparison of the ^{1}H-NMR spectrum and mixed melting point determination with an authentic specimen.

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