[Chem. Pharm. Bull. 28(10)3145—3149(1980)]

Novel Biflavonoids, Chalcan-flavan Dimers from Gambir

The homologous series of novel chalcan-flavan dimers, gambiriin A_1, A_2, A_3, B_1, B_2 and B_3, along with a proanthocyanidin dimer, gambiriin C (epiafzelechin-catechin), have been isolated from Gambir.

Keywords——tannin; Gambir; *Uncaria gambir*; Rubiaceae; chalcan-flavan dimer; proanthocyanidin; astringency

Little chemical research on tannins of Gambir, the dried aqueous extract of the leaves and young twigs of *Uncaria gambir* Roxb. (Rubiaceae), has been made, except for the isolation of (+)-, (±)-catechin and (+)-epicatechin.[1] Our continuative studies on the biologically active polyphenols from crude drugs have led to the isolation of seven new biflavonoids, gambiriin A_1, A_2, A_3, B_1, B_2, B_3 and C, from Gambir. This communication concerns the structure elucidation of these compounds.

Gambiriin A_1 (I), an astringent non-crystalline powder, $[\alpha]_D$ −14.2° (acetone), does not form an anthocyan pigment on treatment with mineral acids. The proton magnetic resonance (PMR) spectrum of I reveals the presence of four benzylic methylene protons (δ 2.40—3.10) and one phloroglucinol group (δ 5.94, 2H, singlet), in addition to seven aromatic protons among which one proton is shifted to the higher field (δ 6.10, singlet), characteristic of 4,8′- or 4,6′-linked biflavonoids.[2] Methylation of I with dimethyl sulfate and K_2CO_3 in dry acetone afforded a nonamethyl ether (II), a colorless amorphous powder, $[\alpha]_D$ −128.5° (acetone), which exhibits the molecular ion peak at *m/e* 706 and intense peaks at *m/e* 181, 211, 315 (base peak), 327, 345 and 496 on the mass spectrum (MS) (Chart 1). II was easily converted into a crystalline compound (III), $C_{38}H_{42}O_{11}\cdot 1/2H_2O$, mp 101—103°, $[\alpha]_D$ −11.3° (acetone), on treatment with *p*-toluenesulfonic acid in benzene. The PMR spectrum of III suggests the presence of the catechin unit in the molecule, revealing the typical two double doublet signals (δ 2.56, J=8, 16 Hz; δ 3.02, J=6, 16 Hz) and the doublet signal (δ 4.62, J=8 Hz) due to the C_4-methylene protons and the C_2-proton of the catechin moiety, respectively.

Although I afforded (+)-catechin and many other phenolic products on treatment with mineral acids, reaction of I with benzyl mercaptan and ethanolic 0.2 N HCl smoothly cleaved the monomer–monomer linkage to give (+)-catechin and a thioether (IV), a hygroscopic amorphous powder, $C_{22}H_{22}O_6S$, $[\alpha]_D$ +104.0° (acetone). The structure of IV was established by the PMR spectrum which shows the signals of a phloroglucinol group (δ 5.93, 2H, singlet), a hydroxyl-bearing proton (δ 4.04, multiplet) and a benzylic methine proton with a sulphur atom (δ 3.79, doublet, J=6 Hz), along with a broad singlet signal (δ 7.24) of five aromatic protons and an AB-type quartet signal (δ 3.59, J=14 Hz) attributed to the benzylthio group. Cleavage reaction of the monomer–monomer linkage in proanthocyanidin dimers with benzyl mercaptan under acid catalysis was reported to proceed stereospecifically,[3] yielding with retention of configuration, the corresponding thioether from the upper half and the free flavan-3-ol from the lower half of the dimer. Regiospecific desulphurization–cyclization reaction was achieved by the usual methylation procedure (dimethyl sulfate and K_2CO_3 in dry acetone)[4] to give (+)-catechin tetramethyl ether, mp 145—146°, $[\alpha]_D$ −11.8° ($CHCl_3$). Thus the absolute stereochemistry of the two asymmetric centers in the upper chalcan unit is concluded to be the same configuration as that of (+)-catechin (2*R*, 3*S*-configuration).

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3) R.S. Thompson, D. Jacques, E. Haslam, and R.J.N. Tanner, *J. Chem. Soc. Perkin I*, **1972**, 1387.
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The ABX-type aromatic signal due to the lower half is observed on the PMR spectrum of III, in relatively higher field (δ 6.52—6.75) as compared with that of XI (δ 6.83—7.06) whose structure is discussed later. The upfield shift is presumably due to the anisotropic effect of the catechol ring in the upper half. On the basis of these data, the upper half unit was determined to be linked to the 8-position of the lower catechin unit.

Gambiriin A_2 (V), a light tan amorphous powder, $[\alpha]_D$ +66.8° (acetone), yielded a nonamethyl ether (VI), a colorless amorphous powder, $[\alpha]_D$ −40.3° (acetone). The MS of VI dis-

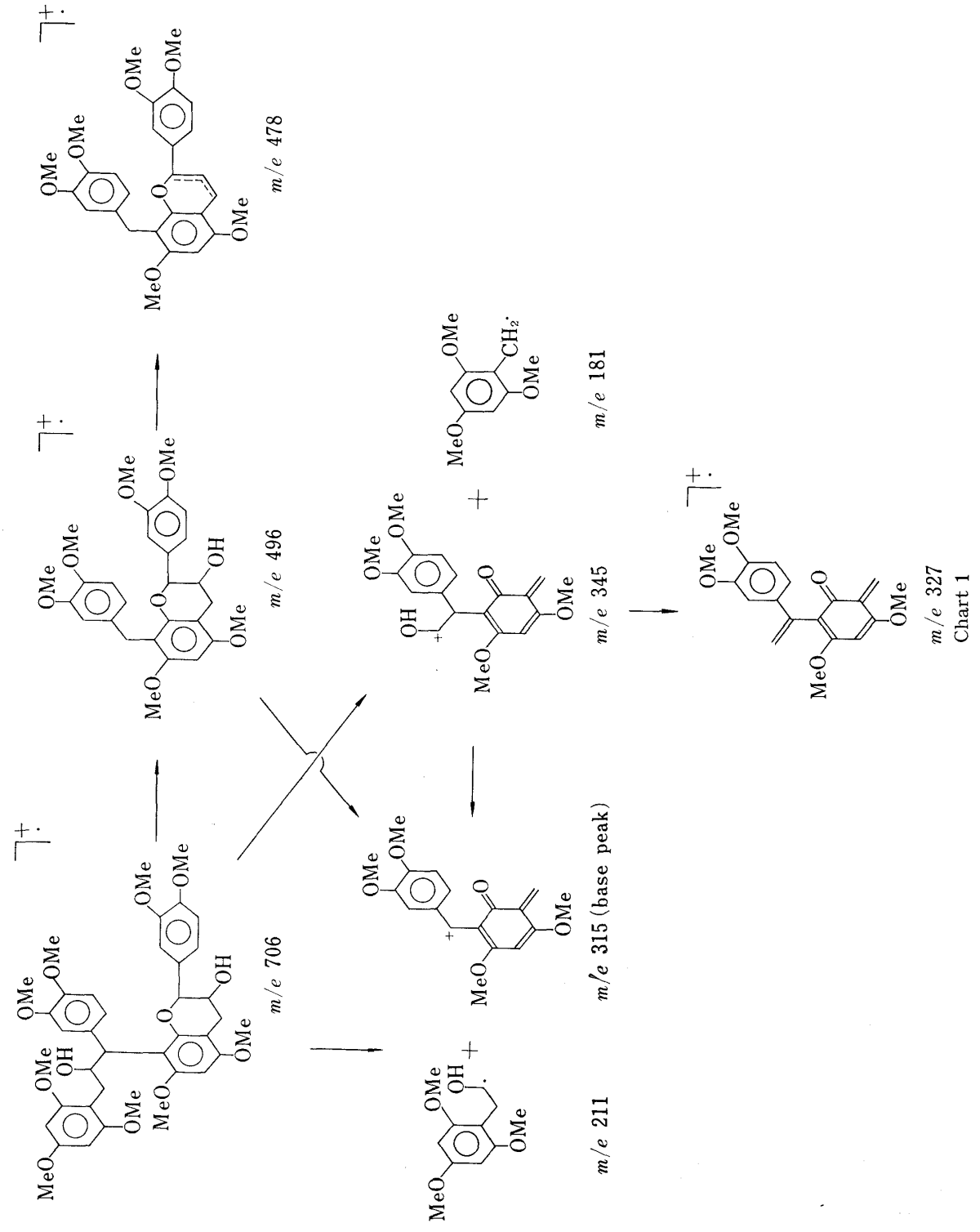

Chart 1

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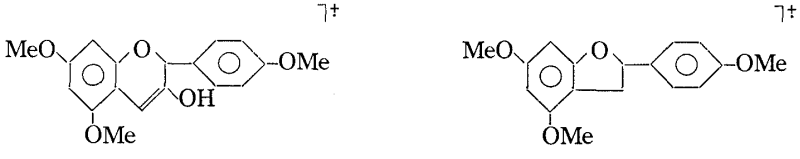
closes the similar fragmentations with that of II, having the same molecular ion peak at *m/e* 706. VI exhibits, in analogy with II, the duplicated proton signals on the PMR spectrum because of the restricted rotation about the interflavan linkage.[3] Treatment of VI with *p*-toluenesulfonic acid in benzene gave colorless crystals (VII), mp 191—192°, $[\alpha]_D$ +109.4° (acetone), which exhibits diagnostic signals ascribable to the C_4-methylene protons (δ 2.90, doublet, J=4 Hz) and C_2-methine proton (δ 4.75, singlet) of the lower half unit, suggesting the presence of epicatechin.

Treatment of V with benzyl mercaptan and ethanolic 0.2 N HCl gave a thioether and (+)-epicatechin. The PMR spectra and optical rotations of these compounds are completely identical with those of IV and the authentic specimen, respectively.

The position of the monomer–monomer linkage was deduced to be 8-position in the epicatechin unit from the PMR spectrum of VII which shows the close resemblance of chemical shift and splitting pattern in the aromatic region (δ 6.14—6.92) with that of III.

Gambiriin A_3 (VIII), a light tan amorphous powder, $[\alpha]_D$ −1.2° (acetone), gives a closely related PMR spectrum with I. On methylation with dimethyl sulfate and K_2CO_3, VIII formed a colorless nonamethyl ether (IX), $[\alpha]_D$ −11.8° (acetone), which shows essentially the same MS as II with the parent peak at *m/e* 706 and the base peak at *m/e* 315. Thus VIII is suggested to be a structural isomer of I. Treatment of IX with *p*-toluenesulfonic acid in benzene afforded (+)-catechin tetramethylate (the optical rotatory dispersion confirms it is racemic), and a trace amount of XI, a colorless amorphous powder, $[\alpha]_D$ +6.0° (acetone), indicating that the chalcan unit is located at the C_6-position of the catechin unit.

Gambiriin B_3 (X), colorless needles, $C_{30}H_{26}O_{11}\cdot 3H_2O$, mp 271—274° (decomp), $[\alpha]_D$ −20.0° (MeOH), shows a bluish purple color with $FeCl_3$ reagent and does not possess an astringency. On the PMR spectrum of X is easily recognized the presence of the catechin unit by the signals at δ 2.61, 2.94 (each double doublet, J=8, 14 Hz; J=5, 14 Hz, respectively, C_4-2H), δ 4.06 (multiplet, C_3–H) and δ 4.57 (doublet, J=7 Hz, C_2–H). Three additional signals observed at δ 3.03 (2H, doublet, J=7 Hz), δ 4.38 (1H, doublet, J=4 Hz) and δ 4.82 (1H, multiplet) in the aliphatic field, due to two methylene protons, a benzylic methine proton and a proton attached to the carbon with an oxygen function, respectively, along with ABX-type signals at δ 6.25 (double doublet, J=2, 8 Hz), δ 6.42 (doublet, J=2 Hz) and δ 6.62 (doublet, J=8 Hz), and a singlet signal at δ 6.02 (2H), in the aromatic proton region, suggest the existence of the chalcan unit with the same oxygenated patterns as that of I. Methylation of X with dimethyl sulfate and K_2CO_3 yielded an octamethyl ether (XI), a colorless amorphous powder, $[\alpha]_D$ +4.8° (acetone). The MS of XI discloses the molecular ion at *m/e* 674, and the base peak at *m/e* 181 which is presumably attributed to the phloroglucinol derivative (Chart 1). XI gave a monoacetate (XII), a colorless amorphous powder, $C_{40}H_{44}O_{12}$, with acetic anhydride and pyridine. The PMR spectrum of XII exhibits signals of an acetyl group at δ 1.97, and an acetoxyl-bearing C_3-proton in the catechin unit at δ 5.41 which is shifted to the low field. Another proton signal bearing an oxygen atom remains unchanged (δ 4.79). These observations, coupled with the formation of an octamethyl ether, strongly suggest the chalcan unit is combined with the residual catechin unit through the ether and carbon–carbon linkages. The position of these linkages were determined by the presence of the unaffected signal (δ 6.22) due to the C_8-proton of the catechin unit on the PMR spectrum of a nonaacetate (XIII) of X ($\Delta\delta$(XII, XIII) 0.07). The C_6-proton



m/e 314 *m/e* 286

Chart 2

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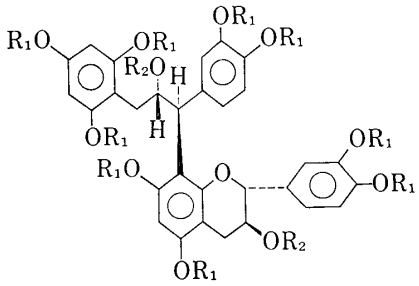
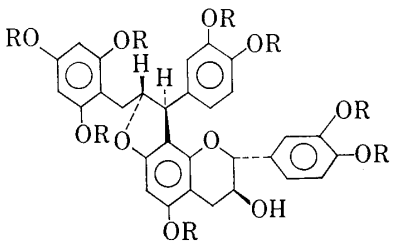
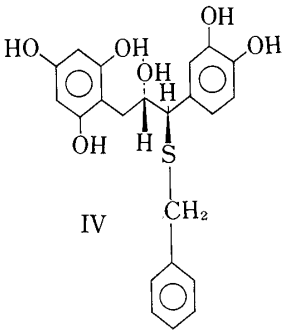


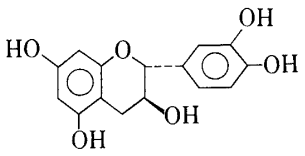
I : $R_1=R_2=H$
II : $R_1=Me$, $R_2=H$
XIV : $R_1=R_2=Ac$
XV : $R_1=Me$, $R_2=Ac$



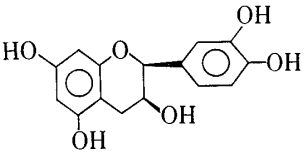
III : R=Me
XVI : R=H



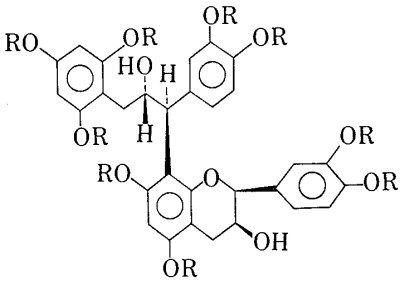
IV



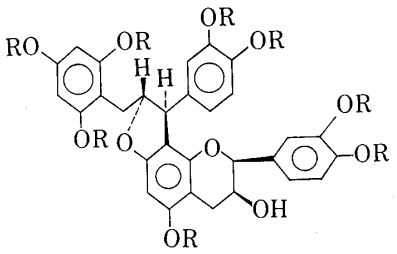
(+)-catechin



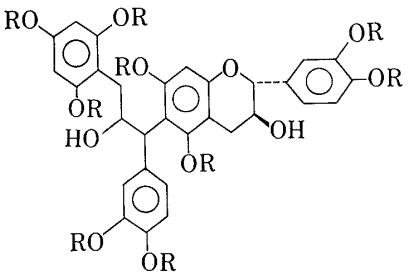
(+)-epicatechin



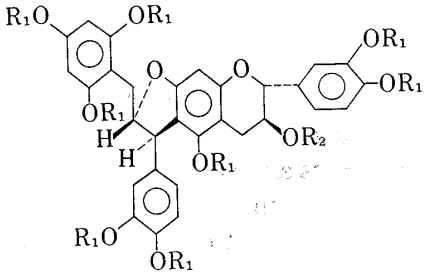
V : R=H
VI : R=Me



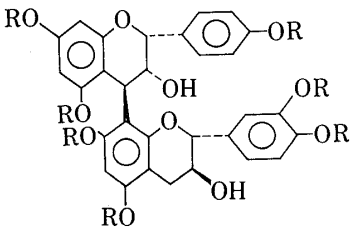
VII : R=Me
XVII : R=H



VIII : R=H
IX : R=Me



X : $R_1=R_2=H$
XI : $R_1=Me$, $R_2=H$
XII : $R_1=Me$, $R_2=Ac$
XIII : $R_1=R_2=Ac$



XVIII : R=H
XIX : R=Me

Chart 3

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signal of a undecaacetate (XIV) derived from I appeared at δ 6.59 ($\Delta\delta$ (XIV, XV) 0.50) shifted to fairly low field.

The stereochemistry of dihydrobenzofuran group was deduced from the PMR spectrum of XI, which reveals almost the same chemical shifts (δ 4.29, doublet; δ 4.82, multiplet) and coupling constants (J=4 Hz) ascribable to the dihydrobenzofuran as those of III (δ 4.22, J= 4 Hz; δ 4.83, multiplet) and VII (δ 4.34, J=4 Hz; δ 4.85, multiplet), indicating that the dihydrobenzofuran ring in XI probably has the *trans*-configuration, similarly as that of III.

Gambiriin B_1 (XVI), colorless needles, mp 183—186°, $[\alpha]_D$ −45.1° (acetone), showing a bluish purple color with $FeCl_3$ reagent similarly as gambiriin B_3, formed an octamethyl ether on methylation with dimethyl sulfate and K_2CO_3. The PMR spectrum of this methylate reveals characteristic signals for the chalcan–catechin dimer, and is identical with that of III.

Gambiriin B_2 (XVII) was isolated from the mother liquor of XVI as its methyl ether whose PMR spectrum was coincided with that of VII.

Gambiriin C (XVIII), a light tan amorphous powder, $[\alpha]_D$ +6.0° (acetone), forms an orange-red color with ethanolic HCl, characteristic of proanthocyanidin,[5] giving a mixture of catechin and pelargonidin. The PMR spectrum of XVIII reveals almost the same signals as that of procyanidin B-1, the 4,8′-linked epicatechin–catechin dimer, which was isolated from *Vaccinium vitis-idaea* L.,[3] apart from the A_2B_2-type aromatic proton signals. Methylation of XVIII with dimethyl sulfate and K_2CO_3 in dry acetone yielded a colorless heptamethyl ether (XIX), $[\alpha]_D$ +114.6° (acetone). The MS of XIX shows the parent peak at *m/e* 660 and peaks at *m/e* 479 and 462 which are probably formed by the *retro*-Diels–Alder type cleavage in the lower flavan unit. The prominent peaks at *m/e* 314 and 286 suggest the upper flavan unit to be epiafzelechin. Thus gambiriin C is characterized to be epiafzelechin-catechin dimer and formulated as XVIII.

Gambiriin A_1, A_2, A_3, B_1, B_2 and B_3 represent a novel type of biflavonoids with the chalcan unit in the molecule, and it is interesting that the gambiriin A group has an astringency, while the B group does not. Biological tests of these compounds are in progress.

Acknowledgement The authors are grateful to Prof. H. Ageta of Showa College of Pharmacy for the measurements of CD spectra, and to Dr. Y. Kawazoe of this faculty for valuable discussions on the sulphur chemistry. Thanks are also due to the staff of central analysis room of this faculty for PMR, CMR and mass spectral measurements.

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Received June 18, 1980

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