Bioactive Flavonoids of the Flowers of *Butea monosperma*

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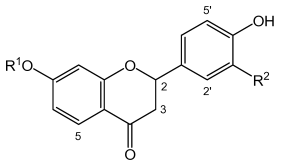
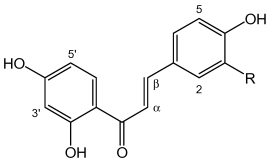
One new dihydrochalcone, dihydromonospermoside (7), was isolated from the flowers of *Butea monosperma* together with three known chalcones, butein (2), monospermoside (4) and isoliquiritigenin (8), one flavone, 7,3′,4′-trihydroxyflavone (6), four flavanones, (−)-butin (1a), (−)-butrin (3a), (+)-isomonospermoside (5b) and (−)-liquiritigenin (9a), and three isoflavones, formononetin (10), afrormosin (11) and formononetin-7-*O*-β-D-glucopyranoside (12). The structure of the new compound was elucidated by spectroscopic techniques whereas those of the known compounds were identified by comparisons of spectroscopic and some physical data with those of reported compounds. The absolute configurations at the 2-position of the flavanones 1a, 3a, 5b and 9a were established to be 2*S*, 2*S*, 2*R* and 2*S*, respectively, by circular dichroism spectral measurements and were confirmed by comparison of the optical rotations with those of reported values and by enzymic hydrolysis of the glucosides to the corresponding aglycones. The isolated flavonoids exhibited varying antimycobacterial activity with the chalcone 2 being the most active compound (MIC 12.5 μg/ml).

Key words *Butea monosperma*; flavonoid; circular dichroism; antimycobacterial activity

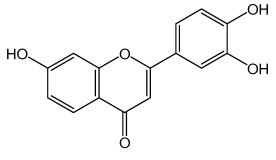
Butea monosperma (Lam.) Taub. (Fabaceae), known in Thai as Tong-kwoaw, is a medium sized deciduous tree of up to 50 feet high with stunning flower clusters, very conspicuous when in flower, and the trunk is usually crooked and twisted with irregular branches and rough, grey bark. The flowers are bright orange red, large and in rigid racemes.[1] This plant species has been found to display a wide variety of biological activities. The stem bark is used in indigenous medicine for the treatment of dyspepsia, diarrhea, dysentery, diabetes, ulcers, sore throat and snake bites.[2] The flower extract showed antidiabetic effect in rats.[3] The plant leaves exhibited antiinflammatory effect[4] and exerted effect on stress, anxiety and cognition in rats.[5] Anti-diarrhoeal activity of the ethanolic extract of the stem bark[6] and anticonvulsive activity of the petroleum ether extract of the flowers[7] of this plant species in experimental animals have been reported. The plant extracts exhibited other biological activities including aphrodisiac activity in male rats,[8] anthelmintic activity,[9,10] and dermal wound healing in rats.[11] Isolation of (−)-medicarpin with antifungal activity from this part of the plant has also been reported.[12] From the flowers of this plant species the flavonoids butin (**1**), butein (**2**), butrin (**3**), isobutrin, palasitrin, coreopsin, isocoreopsin, sulphuresin, monospermoside (**4**), isomonospermoside (**5**) and 7,3′,4′-trihydroxyflavone (**6**) have been isolated.[13—15] Antihepatotoxic activity of the flower extract[16] and of compound **3** and isobutrin[17] has been observed. Compound **1** which was isolated from the seeds of this plant species showed estrogenic and postcoital anti-conceptive activity in rats.[18] A lectin was also isolated from the seeds and was found to agglutinate human erythrocytes. The activity was inhibited by *N*-acetyl galactosamine and did not require a divalent ion.[19] The euphane triterpenoid 3α-hydroxyeuph-25-ene and the alcohol 2,14-dihydroxy-11,12-dimethyl-8-oxo-octadec-11-enylcyclohexane have been isolated from the stem.[20] The imide palasimide has been isolated from the pods of this plant species.[21] Stigmasterol, stigmasterol-3-*O*-β-D-glucopyranoside and nonacosanoic acid were also among those isolated compounds.[15,20] As part of an ongoing project on bioactive compounds from Thai medicinal plants for the treatments of tropical diseases, the plant species was investigated and it was found that the EtOAc and MeOH extracts exhibited antimycobacterial activity. The present report deals with the



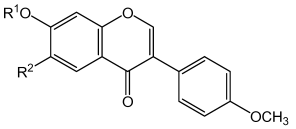
1, R^1 = H, R^2 = OH (Butin)
3, R^1 = Glu, R^2 = OGlu (Butrin)
5, R^1= H, R^2 = OGlu (Isomonospermoside)
9, R^1 = R^2 = H (Liquiritigenin)



2, R = OH (Butein)
4, R = OGlu (Monospermoside)
8, R= H (Isoliquiritigenin)



6, 7,3',4'-Trihydroxyflavone



10, R^1 = R^2 = H (Formononetin)
11, R^1 = H, R^2 = OCH_3 (Afrormosin)
12, R^1= Glu, R^2 = H (Formononetin-7-*O*-β-D-glucopyranoside)

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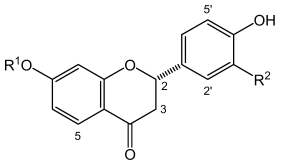
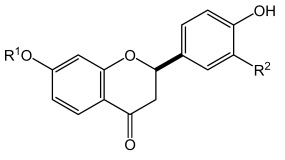


1a, $R^1 = H$, $R^2 = OH$ ((-)-Butin)
3a, $R^1 = Glu$, $R^2 = OGlu$ ((-)-Butrin)
9a, $R^1 = R^2 = H$ ((-)-Liquiritigenin)

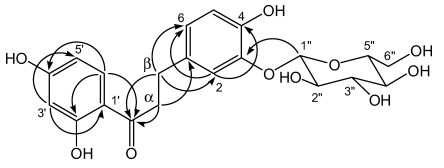


1b, $R^1 = H$, $R^2 = OH$ ((+)-Butin)
5b, $R^1 = H$, $R^2 = OGlu$ ((+)-Isomonospermoside)

isolation and antimycobacterial activity of the isolated new compound **7** and the known compounds **1a**, **2**, **3a**, **4**, **5b**, **6**, **8**, **9a**, and **10**—**12**.

Investigation of the flowers of *Butea monosperma* resulted in the isolation of twelve flavonoids. These included a new dihydrochalcone, dihydromonospermoside (**7**), together with three known chalcones, butein (**2**),[13] monospermoside (**4**)[14,22] and isoliquiritigenin (**8**),[23,24] one flavone, 7,3′,4′-trihydroxyflavone (**6**),[25] four flavanones, (−)-butin (**1a**),[26,27] (−)-butrin (**3a**),[17,27] (+)-isomonospermoside (**5b**)[14] and (−)-liquiritigenin (**9a**),[23] and three isoflavones, formononetin (**10**),[28] afrormosin (**11**)[29] and formononetin-7-*O*-*β*-D-glucopyranoside (**12**).[29] The spectroscopic and physical data of the flavanones **1a**, **3a**, **5b** and **9a** are presented in Experimental. The structure of the new compound was elucidated by spectroscopic techniques whereas those of the known compounds were identified by comparisons of spectroscopic data with those of reported compounds. The absolute configurations of the isolated flavanones **1a**, **3a**, **5b** and **9a** have been determined by circular dichroism (CD) and were confirmed by comparison of the optical rotations with those of reported values and by enzymic hydrolysis of the glucosides to the corresponding aglycones.

Compound **7** was obtained as pale yellow gum, $[\alpha]_D^{26}$ −33.9° (c=0.15, MeOH). The IR spectrum showed absorption for the hydroxyl (3385 cm^{-1}) and a chelated aromatic keto (1636 cm^{-1}) groups. The electrospray-time of flight (ES-TOF)-MS (positive ion mode) gave an $[M+Na]^+$ ion at m/z 459.1269, corresponding to a molecular formula of $C_{21}H_{24}O_{10}$. The ^{1}H-NMR spectrum of compound **7** exhibited an *α*,*β*-saturated keto function as a triplet-like signal (J=7.1 Hz) of two *β*-protons at *δ* 2.88 and a multiplet signal of two *α*-protons at *δ* 3.07. The 2′,4′-dihydroxyl substituted pattern was confirmed by heteronuclear multiple bond connectivity (HMBC) correlation as shown in Fig. 1. Placement of the 2′-hydroxyl group was confirmed by the presence of a chelated signal at *δ* 12.80 in the ^{1}H-NMR spectrum of **7**. The ^{1}H-NMR spectrum of **7** revealed ABX systems in both the A- and B-rings at *δ* 6.25 (br s, H-3′), 6.27 (br dd, J=8.9, 2.3 Hz, H-5′), and 7.47 (d, J=8.9 Hz, H-6′); and *δ* 6.84 (br s, H-2), 6.76 (apparent br s, H-5 and H-6). Upon addition of C_6D_6, the apparent broad singlet signal of H-5 and H-6 resolved to two doublets (J=8.1 Hz) at *δ* 6.88 (d) and 6.84 (br d), respectively, whereas the H-2 signal appeared as a broad singlet signal at *δ* 6.99. The fourth substituent was a



7, Dihydromonospermoside

HMBC correlation

Fig. 1. Structure and Selected HMBC Correlations of Dihydromonospermoside (**7**)

glucosidic moiety as evident from the presence of the anomeric proton at *δ* 4.50 (d, J=7.2 Hz, H-1″), and other proton signals at *δ* 3.36—3.50 (m, H-2″, H-3″ and H-4″), 3.29 (m, H-5″), 3.73 (dd, J=12.1, 4.0 Hz, H-6″a) and 3.78 (dd, J=12.1, 2.9 Hz, H-6″b). The anomeric proton signal of H-1″ attributable to that of *β*-glucosyl unit from the coupling constant. The ^{1}H- and ^{13}C-NMR data of **7** were similar to those of monospermoside (butein-3-*O*-glucopyranoside, **4**)[22]; the significant differences being the presence of two methylene signals at *δ* 2.88 and *δ* 3.07, and the absence of olefinic protons of the *α*,*β*-unsaturated keto system. Assignments of the ^{1}H- and ^{13}C-NMR signals were confirmed by 1D and 2D NMR techniques (see also the selected HMBC correlations in Fig. 1). The point of attachment of the 1-position of the glucosidic moiety to the 3-position of the dihydrochalcone was established by HMBC correlation (see Fig. 1). The ROESY spectrum showed cross peak between H-2 and H-1″ confirming the existence of C-3–*O*–C-1″ linkage. The structure of compound **7** was thus concluded as the *α*,*β*-saturated analogue of monospermoside (**4**) and named dihydromonospermoside.

The absolute configuration at C-2 of flavanones could not be determined from the coupling constants of H-2 and H-3 in the ^{1}H-NMR spectrum, since natural flavanones exist in the thermodynamically favoured conformation with the C-2 aryl substituent in the equatorial orientation and it follows that $J_{2,3ax}$ for both *S* and *R* isomers are large.[30] The C-2 configuration of flavanones was determined by CD spectral measurement.[31,32] The UV absorptions of flavanones at 270—290 nm and 320—330 nm have been assigned to the *π*→*π** and n→*π** acetophenone chromophore transitions, respectively. Flavanones with 2*S* configuration possessing a conformation with *P*-helecity of the heterocyclic ring and having a C-2 equatorial aryl group exhibit a positive Cotton effect at the n→*π** absorption band and a negative Cotton effect at the *π*→*π** absorption band.[32] The absolute configurations at C-2 of the isolated flavanones **1a**, **3a**, **5b** and **9a** were established by CD spectral measurements[31,32] and comparison of the specific rotations with those of the reported compounds. The C-2 aryl substituent of the flavanone (−)-butin (**1a**) was in the equatorial orientation, as evident from the large $J_{2,3ax}$ (12.5 Hz). The CD spectrum of **1a** showed negative Cotton effect at the *π*→*π** absorption band ($[\theta]_{291}$ −11600), which indicated the 2-position to be the *S* orientation. The specific rotation of **1a** ($[\alpha]_D^{26}$ −39.9° (c=0.40, MeOH) was comparable to the reported value ($[\alpha]_D^{22}$ −36.1° (c=1.0, MeOH)) of (−)-butin.[27] The C-2 configuration of flavanone glycosides can be determined in the same manner to that of the aglycone.[32] The CD spectra of (−)-butrin (**3a**) showed positive Cotton effect at the n→*π** absorption band ($[\theta]_{340}$ +83327),

Table 1. Antimycobacterial Activity of the Isolated Flavonoids

Compound	MIC[a,b] (μg/ml)
1a	25
2	12.5
3a	50
4	25
5b	25
6	50
7	50
8	25
9a	25
10	50
11	25
12	100

a) MIC at >200 μg/ml is regarded as inactive. *b*) The standard drugs, kanamycin sulphate, isoniazid and rifampicin, showed MIC values of 2.5, 0.06 and 0.004 μg/ml, respectively.

which indicated the 2*S* configuration. The specific rotation of **3a** was also comparable to the reported value of (−)-butrin.[27] It should be noted that, though it was established that the laevorotatory flavanones possess a 2*S* configuration,[33] the optical rotation of the corresponding glycosides does not necessarily follow this trend and this was due to the chirality of the carbohydrate moiety.[32] The C-2 configuration of **3a** was further confirmed by enzymic hydrolysis of **3a** with β-glucosidase to (−)-butin (**1a**) (see Experimental). Flavanones with 2*R* configuration with a C-2 equatorial aryl group exhibit a negative Cotton effect at the n→π* absorption band and a positive Cotton effect at the $\pi\rightarrow\pi$* absorption band.[32] The CD spectra of (+)-isomonospermoside (**5b**) showed negative maximum in the long wavelength region ($[\theta]_{342}$ −55403), which indicated the 2*R* configuration. The specific rotation of **5b** ($[\alpha]_D^{26}$ +6.4° (*c*=0.30, MeOH) was not compared with the literature value, since no report has been found. The configuration of **5b** was confirmed by enzymic hydrolysis to (+)-butin (**1b**) (see Experimental). The configuration of **1b** was determined to be *R* from the negative Cotton effect at the n→π* absorption band ($[\theta]_{345}$ −20156). Compound **1b** gave positive specific rotation with $[\alpha]_D^{26}$ +19.0° (*c*=0.05, MeOH). The *S* configuration of compound **9a** was similarly determined from CD spectral measurement ($[\theta]_{280}$ −6558). It should be noted that the $J_{2,3ax}$ of the 2*R* flavanones **5b** and **1b** are approximately the same order of magnitude as those of the 2*S* flavones **1a** and **9a** (see Experimental).

All the isolated flavonoids were subjected to antimycobacterial evaluation and the results are presented in Table 1. The chalcone butein (**2**) exhibited highest activity with MIC of 12.5 μg/ml. The activity of the other flavonoids was between 25 and 100 μg/ml. The relationships between the flavonoid structures and their antimycobacterial activity could not be deduced from the existing data. However, the assay results indicated that the presence of glucosidic moiety tended to decrease the activity of the flavonoid aglycone and that the absence of α,β-olefinic system decreased the biological activity.

Experimental

General Procedures Melting points were determined with an Electrothermal melting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO-1020 digital polarimeter and CD spectra were recorded in methanol on a JASCO J-810 spectropolarimeter. IR spectra were recorded in KBr on a Perkin-Elmer FT-IR Spectrum BX spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker AVANCE 400 FT-NMR spectrometer, operating at 400 (^{1}H) and 100 (^{13}C) MHz. For the spectra taken in CD_3OD, $CDCl_3$ and DMSO-d_6 the residual nondeuterated solvent signals at δ 3.30, 7.24 and 2.49, and the solvent signals at δ 49.0, 77.0 and 39.5, were used as references for ^{1}H- and ^{13}C-NMR spectra, respectively. ES-TOF-MS and ES-MS spectra were measured with a Bruker micrOTOF and a Finnigan LC-Q mass spectrometer. Unless indicated otherwise, column chromatography and TLC were carried out using Merck silica gel 60 (finer than 0.063 mm) and precoated silica gel 60 F_{254} plates, respectively. Spots on TLC were detected under UV light and by spraying with anisaldehyde–H_2SO_4 reagent followed by heating.

Plant Material The flowers of *B. monosperma* were purchased from Tai-un-jan herbal store, Bangkok in 2006. A voucher specimen is deposited at the Faculty of Science, Ramkhamhaeng University (Apichart Suksamrarn, No. 054).

Extraction and Isolation The dried flowers (15.0 kg) were milled and soaked successively with *n*-hexane, EtOAc and MeOH. The hexane, EtOAc and MeOH extracts were evaporated under reduced pressure to give the crude hexane extract (brownish syrup, 32.8 g), the EtOAc extract (dark brownish sticky solid, 102.7 g) and the MeOH extract (dark brownish sticky solid, 527.6 g).

The EtOAc extract (100.0 g) was fractionated by quick column chromatography (Merck silica gel 60 PF_{254}, 500 g), eluting with *n*-hexane, *n*-hexane–EtOAc and EtOAc with increasing amounts of the more polar solvent. The eluates were examined by TLC and 10 combined fractions (C1—C10) were obtained. Fraction C7 was rechromatographed over silica gel (0.063—0.200 mm, 125 g) with *n*-hexane–EtOAc and EtOAc as eluting solvent to give 6 subfractions. Subfraction 3 was chromatographed eluting under isocratic condition (20% EtOAc in *n*-hexane) to yield formononetin (**10**)[28] as orange solid (10.4 mg), isoliquiritigenin (**8**)[23,24] as pale yellow solid (6.8 mg) and afrormosin (**11**)[29] as white solid (11.0 mg). The position of the methoxyl group of compound **10** was confirmed by HMBC correlation between the methoxyl proton signal with C-4′. The positions of the two methoxyl groups of compound **11** were at the 6- and 4′-positions from NOE experiments of the 6-OMe and H-5, and of the 4′-OMe and H-3′/H-5′, respectively. Subfraction 4 was chromatographed using *n*-hexane–EtOAc and EtOAc as eluents, with increasing amount of the more polar solvent, to afford liquiritigenin (**9a**)[23] (34.7 mg). Fraction C9 was combined and chromatographed over silica gel (0.063—0.200 mm, 500 g) using CH_2Cl_2 and CH_2Cl_2–MeOH as eluents, with an increasing amount of the more polar solvent, to give 3 subfractions. Subfraction 2 was chromatographed over silica gel and eluted under isocratic condition (2% MeOH in CH_2Cl_2) to give butin (**1a**).[14,26] Subfraction 3 was subjected to repeated column chromatography (3×) with similar eluting solvent systems to give butein (**2**)[13] as orange solid (270.0 mg).

The MeOH extract (520.0 g) upon standing white solid separated out, which was recrystallized from MeOH and CH_2Cl_2 to give 42.3 g of (−)-butrin (**3a**).[14,17] The ^{1}H- and ^{13}C-NMR data were consistent with the reported values.[27] The mother liquor was evaporated and the residue (475.5 g) was chromatographed over silica gel (0.063—0.200 mm, 650 g) eluting with CH_2Cl_2, CH_2Cl_2–MeOH and MeOH with increasing amount of the more polar solvent. The eluates were examined by TLC and 13 groups of eluting fractions (fractions M1—M13) were obtained. Fraction M8 was chromatographed over silica gel (0.063—0.200 mm, 30 g) and eluted under isocratic condition (2% MeOH in CH_2Cl_2) to give 7,3′,4′-trihydroxyflavone (**6**)[25] (4.5 mg).

Fraction M9 was chromatographed over silica gel (0.063—0.200 mm, 30 g) and eluted under isocratic condition (2% MeOH in CH_2Cl_2) to give 11 subfractions. Subfraction 6 was rechromatographed over silica gel and eluted under isocratic condition (3% MeOH in CH_2Cl_2) to give formononetin-7-*O*-β-D-glucopyranoside (**12**)[29] as white amorphous solid (5.2 mg). The position of the methoxyl group was confirmed by HMBC correlation of the methoxyl proton signal with C-4′. Subfraction 8 was rechromatographed twice eluting with CH_2Cl_2 and CH_2Cl_2–MeOH with increasing amount of the more polar solvent to give (+)-isomonospermoside (**5b**)[14] as white amorphous solid (104.5 mg), dihydromonospermoside (**7**) as pale yellow gum (61.2 mg) and monospermoside (**4**)[14,22] as yellow amorphous solid (596.4 mg).

(−)-Butin (**1a**): White amorphous solid, $[\alpha]_D^{26}$ −39.9° (*c*=0.40, MeOH) (lit.[27] $[\alpha]_D^{22}$ −36.1° (*c*=1.0, MeOH)); CD (*c*=0.06, MeOH) $[\theta]^{25}$ (nm): −11600 (291) (negative maximum); IR (KBr) cm^{-1}: 3481, 3366, 3119, 1664, 1607, 1584, 1508, 1361, 1326, 1287, 1266, 1232, 1170, 1113, 994, 819; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.62 (1H, dd, *J*=16.7, 2.9 Hz, H-

3eq), 3.03 (1H, dd, J=16.7, 12.5 Hz, H-3ax), 5.36 (1H, dd, J=12.5, 2.9 Hz, H-2), 6.32 (1H, d, J=2.1 Hz, H-8), 6.47 (1H, dd, J=8.7, 2.1 Hz, H-6), 6.72 (2H, s-like, H-5′, H-6′), 6.86 (1H, s, H-2′), 7.61 (1H, d, J=8.7 Hz, H-5), 9.02 (2H, brs, OH); ^{1}H-NMR (400 MHz, $CDCl_3$+5 drops CD_3OD) δ: 2.67 (1H, dd, J=16.9, 2.9 Hz, H-3eq), 2.93 (1H, dd, J=16.9, 12.9 Hz, H-3ax), 5.23 (1H, dd, J=12.9, 2.9 Hz, H-2), 6.31 (1H, d, J=2.1 Hz, H-8), 6.44 (1H, dd, J=8.7, 2.1 Hz, H-6), 6.73 (1H, dd, J=8.1, 1.6 Hz, H-6′), 6.78 (1H, d, J=8.1 Hz, H-5′), 6.87 (1H, d, J=1.6 Hz, H-2′), 7.70 (1H, d, J=8.7 Hz, H-5); ES-MS m/z: 271 $[M-H]^-$.

Butein (**2**): Orange solid; IR (KBr) cm^{-1}: 3301, 2928, 1638, 1558, 1457, 1352, 1235, 1175, 859. ^{1}H-NMR (400 MHz, CD_3OD) δ: 6.28 (1H, d, J=2.4 Hz, H-3′), 6.40 (1H, dd, J=8.8, 2.4 Hz, H-5′), 6.81 (1H, d, J=8.4 Hz, H-3), 7.10 (1H, dd, J=8.4, 1.6 Hz, H-2), 7.17 (1H, d, J=1.6 Hz, H-6), 7.52 (1H, d, J=15.4 Hz, H-α), 7.71 (1H, d, J=15.4 Hz, H-β), 7.92 (1H, d, J=8.8 Hz, H-6′). ES-MS m/z: 567 $[2M+Na]^+$.

(−)-Butrin (**3a**): White solid, mp 193—194 °C from MeOH–CH_2Cl_2 (lit.[27] 190—191 °C); $[\alpha]_D^{26}$ −65.3° (c=0.45, MeOH) (lit.[27] $[\alpha]_D^{21}$ −73.0° (c=1.0, pyridine)); CD (c=0.21, MeOH) $[\theta]^{25}$ (nm): +83327 (340) (positive maximum); IR (KBr) cm^{-1}: 3367, 2921, 2873, 1669, 1612, 1575, 1520, 1442, 1279, 1252, 1191, 1170, 1109, 1073, 1038, 1014; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.67 (1H, dd, J=16.8, 2.6 Hz, H-3eq), 3.20 (1H, obscured signal, H-3ax), 3.15 (2H, m, H-5″, H-5‴), 3.25 (2H, obscured signal, H-2″, H-2‴), 3.40 (4H, obscured signal, H-3″, H-4″, H-3‴, H-4‴), 3.43 and 3.68 (each 2H, each m, H-6″, H-6‴), 4.54 and 4.59 (each 1H, each dd, J=5.5, 5.4 Hz, OH-6″, OH-6‴), 4.71 (1H, d, J=7.1 Hz, H-1‴), 4.97 (1H, d, J=7.2 Hz, H-1″), 5.02 (2H, d, J=4.6 Hz, OH-2″, OH-2‴), 5.09 (2H, br d, J=4.6 Hz, OH-4″, OH-4‴), 5.35 (1H, br d, J=4.1 Hz, OH-3″), 5.45 (1H, obscured signal, OH-3‴), 5.45 (1H, dd, J=12.8, 2.6 Hz, H-2), 6.60 (1H, d, J=2.2 Hz, H-8), 6.70 (1H, dd, J=8.7, 2.2 Hz, H-6), 6.83 (1H, d, J=8.2 Hz, H-5′), 7.02 (1H, dd, J=8.2, 1.5 Hz, H-6′), 7.30 (1H, d, J=1.5 Hz, H-2′), 7.70 (1H, d, J=8.7 Hz, H-5), 8.78 (1H, br s, OH-4′); ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 42.9 (C-3), 60.4 and 60.7 (C-6″ and C-6‴), 69.4 and 69.8 (C-5″ and C-5‴), 73.0 and 73.2 (C-2″ and C-2‴), 75.9 and 76.3 (C-3‴ and C-3‴), 76.9 and 77.1 (C-4″ and C-4‴), 79.2 (C-2), 99.6 (C-1′), 101.9 (C-1‴), 103.4 (C-8), 110.9 (C-6), 115.3 and 115.6 (C-2′ and C-5′), 115.7 (C-10), 121.6 (C-6′), 127.8 (C-5), 129.7 (C-1′), 145.0 (C-3′), 147.1 (C-4′), 162.8 (C-7), 163.4 (C-9), 190.4 (C-4); ES-MS m/z: 595 $[M-H]^-$.

(+)-Isomonospermoside (**5b**): White amorphous solid; $[\alpha]_D^{26}$ +6.4° (c=0.30, MeOH); CD (c=0.24, MeOH) $[\theta]^{25}$ (nm): −55403 (342) (negative maximum); IR (KBr) cm^{-1}: 3346, 2914, 2866, 1656, 1609, 1523, 1466, 1335, 1284, 1162, 1119, 1071, 1034, 990, 799; ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.63 (1H, dd, J=16.8, 2.6 Hz, H-3eq), 3.10 (1H, dd, J=16.8, 12.8 Hz, H-3ax), 3.13 (1H, m, H-5″), 3.26—3.34 (3H, m, H-2″—H-4″), 3.46 (1H, m, H-6″a), 3.68 (1H, br d, J=11.1 Hz, H-6″b), 4.69 (1H, d, J=7.2 Hz, H-1″), 5.40 (1H, dd, J=12.8, 2.6 Hz, H-2), 6.33 (1H, d, J=2.0 Hz, H-8), 6.48 (1H, dd, J=8.6, 2.0 Hz, H-6), 6.82 (1H, d, J=8.2 Hz, H-5′), 7.01 (1H, dd, J=8.2, 1.6 Hz, H-6′), 7.26 (1H, d, J=1.6 Hz, H-2′), 7.62 (1H, d, J=8.6 Hz, H-5); ES-MS m/z: 457 $[M+Na]^+$.

Dihydromonospermoside (**7**): Pale yellow gum; $[\alpha]_D^{26}$ −33.9° (c=0.15, MeOH); IR (KBr) cm^{-1}: 3385, 2927, 1636, 1559, 1458, 1374, 1277, 1171, 1133, 1071, 895; ^{1}H-NMR (400 MHz, $CDCl_3$+5 drops CD_3OD) δ: 2.88 (2H, apparent t, J=7.1 Hz, H_2-β), 3.07 (2H, m, H_2-α), 3.29 (1H, m, H-5″), 3.36—3.50 (3H, m, H-2″—H-4″), 3.73 (1H, dd, J=12.1, 4.0 Hz, H-6″a), 3.78 (1H, dd, J=12.1, 2.9 Hz, H-6″b), 4.50 (1H, d, J=7.2 Hz, H-1″), 6.25 (1H, br s, H-3′), 6.27 (1H, br dd, J=8.9, 2.3 Hz, H-5′), 6.76 (2H, br s, H-5, H-6), 6.84 (1H, br s, H-2), 7.47 (1H, d, J=8.9 Hz, H-6′), 12.80 (*ca.* 1H, br s, OH-2′). Upon addition of C_6D_6, the H-5, H-6 and H-2 signals have changed to δ: 6.84 (1H, br d, J=8.1 Hz, H-6), 6.88 (1H, d, J=8.1 Hz, H-5), 6.99 (1H, br s, H-2). ^{13}C-NMR (100 MHz, $CDCl_3$+5 drops CD_3OD) δ: 30.2 (C-β), 39.5 (C-α), 61.3 (C-6″), 69.5 (C-4″), 73.1 (C-2″), 75.9 (C-5″),[a] 76.0 (C-3″),[a] 102.9 (C-3′), 103.6 (C-1″), 108.2 (C-5′), 113.0 (C-1′), 116.3 (C-5),[b] 118.7 (C-2), 124.4 (C-6),[b] 132.3 (C-6′), 132.5 (C-1), 144.7 (C-3), 145.6 (C-4), 164.4 (C-2′),[c] 164.6 (C-4′),[c] 203.9 (CO), [a—c]assignment may be reversed for signals with the same superscript; ES-MS m/z: 895 $[2M+Na]^+$; ES-TOF-MS (positive ion mode) m/z 459.1269 $[M+Na]^+$ (Calcd for $C_{21}H_{24}O_{10}$+Na: 459.1269).

(−)-Liquiritigenin (**9a**): $[\alpha]_D^{26}$ −5.4° (c=0.29, MeOH) (lit.[34] $[\alpha]_D^{26}$ −36.2° (MeOH)); CD (c=0.008, MeOH) $[\theta]^{25}$ (nm): −6558 (280) (negative maximum); IR (KBr) cm^{-1}: 3549, 3481, 3134, 3025, 2922, 2826, 2715, 1661, 1605, 1574, 1518, 1470, 1332, 1266, 1235, 1217, 1159, 1133, 998, 830; ^{1}H-NMR (400 MHz, $CDCl_3$+2 drops CD_3OD) δ: 2.71 (1H, dd, J=16.8, 2.7 Hz, H-3eq), 2.99 (1H, dd, J=16.8, 13.3 Hz, H-3ax), 5.31 (1H, dd, J=13.3, 2.7 Hz, H-2), 6.34 (1H, d, J=2.0 Hz, H-8), 6.49 (1H, dd, J=8.6, 2.0 Hz, H-6), 6.81 (1H, d, J=8.4 Hz, H-3′, H-5′), 7.26 (1H, d, J=8.4 Hz, H-2′, H-6′), 7.86 (1H, d, J=8.6 Hz, H-5); ES-MS m/z: 255 $[M-H]^-$.

Enzymic Hydrolysis of (−)-Butrin (3a) A solution of **3a** (12 mg) in water (4 ml) was treated with β-glucosidase from almonds (Fluka, 2 mg) and the mixture was stirred at ambient temperature for 4 h. Water (10 ml) was added and the mixture was extracted with EtOAc (3×15 ml). The organic phase was evaporated and the residue was passed through a Sephadex LH-20 column eluting with MeOH to yield (−)-butin (5 mg), $[\alpha]_D^{26}$ −44.8° (c=0.30, MeOH), CD (c=0.10, MeOH) $[\theta]^{25}$ (nm): +11909 (341) (positive maximum). ^{1}H-NMR spectrum (in $CDCl_3$+5 drops CD_3OD) were identical to that of the natural **1a**.

Enzymic Hydrolysis of (+)-Isomonospermoside (5b) Compound **5b** (6 mg) was subjected to enzymic hydrolysis in the same manner employed for compound **3a** to yield (+)-butin (**1b**) (2 mg).

(+)-Butin (**1b**): $[\alpha]_D^{26}$ +19.0° (c=0.05, MeOH); CD (c=0.21, MeOH) $[\theta]^{25}$ (nm): −20156 (345) (negative maximum); IR (KBr) cm^{-1}: 3453, 3229, 1660, 1600, 1576, 1533, 1467, 1356, 1281, 1247, 1195, 1160, 1128, 1074, 867; ^{1}H-NMR (400 MHz, $CDCl_3$+5 drops CD_3OD) δ: 2.71 (1H, obscured signal, H-3eq), 2.94 (1H, dd, J=16.9, 12.9 Hz, H-3ax), 5.27 (1H, dd, J=12.9, 2.8 Hz, H-2), 6.33 (1H, d, J=2.2 Hz, H-8), 6.45 (1H, dd, J=8.7, 2.1 Hz, H-6), 6.77 (1H, dd, J=8.1, 1.7 Hz, H-6′), 6.80 (1H, d, J=8.1 Hz, H-5′), 6.89 (1H, d, J=1.7 Hz, H-2′), 7.72 (1H, d, J=8.7 Hz, H-5); ES-MS m/z: 271 $[M-H]^-$.

Antimycobacterial Assay Antimycobacterial activity was assessed against *Mycobacterium tuberculosis* H_{37}Ra using the Microplate Alamar Blue Assay.[35] In our system, the standard drugs, kanamycin sulphate, isoniazid, and rifampicin showed MIC values of 2.5, 0.06, and 0.004 μg/ml, respectively.

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