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BAUHININ, A NEW NITRILE GLUCOSIDE FROM *BAUHINIA CHAMPIONII*

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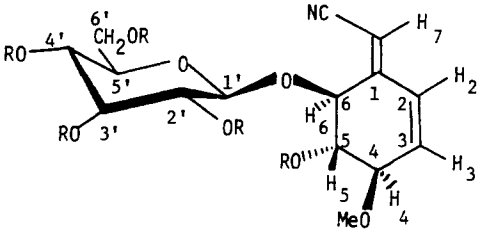
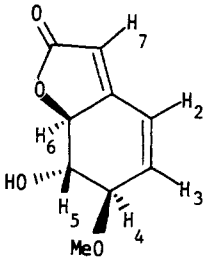
ABSTRACT.—A phytochemical investigation into the roots of *Bauhinia championii* (Leguminosae) has resulted in the isolation and spectral characterization of a new nitrile glucoside, bauhinin ([*Z*]-6β-[β-D-glucosyloxy]-4β-methoxy-5α-hydroxy-2-cyclohexene-$\Delta^{1,\alpha}$-acetonitrile), the structure of which was confirmed by the results of a single-crystal X-ray analysis of its dihydrate. Gallic acid was also isolated from the same source.

Bauhinia championii Benth. (Leguminosae) grows in the mountainous areas and dense forests of Taiwan. In a previous paper (1), we described the isolation and structure determination of six polymethoxyflavones from the roots, which have been reported (2) to possess piscicidal activity. Some of these flavones exhibited inhibitory activity towards ADP- and collagen-induced platelet aggregation (3). Further investigations on the constituents from this same source have now led us to the isolation of a new nitrile glucoside, bauhinin (**1**), in addition to gallic acid (4).

Bauhinin (**1**), M^+ 343, was obtained from H_2O-Me_2CO in the form of colorless prisms of its dihydrate, mp 213-214°. A strong, sharp band at 2220 cm^{-1} in the ir spectrum indicated the presence of a conjugated nitrile (4) in **1**. Other bands at 3450-3250 cm^{-1} and multiple peaks in the 1160-1000 cm^{-1} region were indicative of a



1 R=H
2 R=Ac



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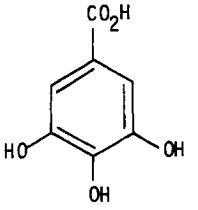

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polyhydroxy compound, while a band at 1630 cm^{-1} suggested the presence of unsaturation. The uv spectrum exhibited strong absorption at λ max 256 nm. Comparison showed that the ir and uv spectra of **1** were very similar to those of griffonin (lithospermoside) (5,6), thus leading to speculation that **1** might be an α,β,γ,δ-unsaturated nitrile. On enzymatic or acidic hydrolysis, **1** failed to produce HCN but yielded an aglycone and D-glucose, thereby demonstrating that it is a glucoside.

Acetylation of **1** yielded pentaacetate (**2**), the ^{1}H-nmr spectrum of which showed five acetoxy groups (δ 2.15, 2.09, 2.04, 2.03, and 2.02 ppm), and, with four of these belonging to the glucose residue, the remaining one must be associated with the aglycone moiety. The nmr spectrum also contained three olefinic proton signals at δ 6.22 (H-2), 6.09 (H-3), and 5.37 (H-7) ppm of which the former two represented the AB part of an ABX system with the X proton (H-4) located at δ 4.02 ppm. The coupling constants between H-4 and H-5 and between H-5 and H-6 indicated a *trans*-diaxial relationship between the protons in each pair. Since the signal for H-5 was significantly shifted downfield compared to those for H-6 and H-4, C-5 must be bonded to a hydroxyl group. The signal centered at δ 4.63 ppm was assigned to the allylic proton, H-6, which is α to the *O*-glycosyl substituent. The low-field value found for this allylic proton, in contrast to that of δ 4.02 ppm for H-4, could be rationalized in terms of a stereoisomeric form wherein the nitrile group is *cis* to the glycosidic linkage and the triple bond would deshield H-6. That the glucose moiety is located at C-6 may be ascertained from the nature of the hydrolysis product (see below). The anomeric proton of the glucose residue appeared at δ 4.84 ppm, and, with a coupling constant of 8 Hz between H-1′ and H-2′, a diaxial relationship was indicated; accordingly, a β-configuration was assigned to the glucose.

Enzymatic or acidic hydrolysis of **1** yielded an aglycone, bauhinilide (**3**), M^+ 182, mp 119-120°. The ir spectrum contained bands at 1770 and 1740 cm^{-1}, indicating the presence of an unsaturated 5-membered lactone ring (4); a band at 1630 cm^{-1} suggested further unsaturation, and C-O stretching frequencies occurred in the 1150-1020 cm^{-1} region. The uv spectrum exhibited strong absorption at λ max 255 nm. These data were consistent with the formulation of an α,β,γ,δ-unsaturated-γ-lactone system. In the ^{1}H-nmr spectrum of **3**, signals for three olefinic protons (H-1, H-2, and H-3) were evident, and H-3, H-2, and H-4 formed an ABX system ($J_{2,3}$=10 Hz, $J_{2,4}$=2 Hz, $J_{3,4}$=2 Hz). There was also a *trans*-relationship between H-4 and H-5 ($J_{4,5}$=8 Hz) as well as between H-5 and H-6 ($J_{5,6}$=8 Hz). The methoxyl group remained at C-4, and so it must have been the oxygen at C-6 that participated in lactone formation. Thus, it was firmly established that the glucose residue in **1** is located at C-6 and the methoxyl group is at C-4.

On the basis of the foregoing data, bauhinin was assigned structure **1**, i.e., [*Z*]-6β-[β-D-glucosyloxy]-4β-methoxy-5α-hydroxy-2-cyclohexene-$\Delta^{1,\alpha}$-acetonitrile. The complete structure was confirmed by the results of a single-crystal X-ray analysis of the dihydrate, and the absolute stereochemistry follows from the known absolute configuration of β-D-glucose.

The crystal structure was solved by direct methods (7). Full-matrix least-squares refinement of atomic positional and thermal parameters converged to R=0.042[1] over 1659 reflections measured by diffractometer. A view of the solid-state conformation, with the atom numbering scheme, is provided in Figure 1. Final atomic positional parameters are in Table 1.

All bond lengths lie close to expected values. The overall geometry of the pyranose ring is very similar to that reported for β-D-glucose (8). Torsion angles in the cyc-

[1] $R=\Sigma||F_o|-|F_c||/\Sigma|F_o|$.

TABLE 1. Non-Hydrogen Atom Fractional Coordinates ($\times 10^5$), with Standard Deviations in Parentheses

Atom	*x*	*y*	*z*
C(1)	40586(21)	7253(19)	51615(44)
C(2)	49256(24)	11571(23)	71798(55)
C(3)	46264(24)	18259(22)	85285(51)
C(4)	34095(23)	22118(19)	80753(43)
C(5)	26615(19)	19834(18)	55287(40)
C(6)	27904(19)	9356(17)	50127(40)
C(7)	44663(22)	1927(22)	36309(51)
C(8)	38041(23)	−2737(22)	15009(50)
N(9)	33913(24)	−6731(21)	−2601(44)
O(10)	34378(20)	32128(14)	83036(33)
C(11)	37282(32)	35438(25)	107236(54)
O(12)	14525(15)	22041(13)	52552(31)
C(1′)	11934(19)	0(−)[a]	27444(40)
C(2′)	4373(19)	−1282(17)	1603(41)
C(3′)	−3447(18)	−9892(17)	−602(41)
C(4′)	3415(19)	−18534(17)	10786(40)
C(5′)	10924(22)	−16361(17)	36175(41)
C(6′)	19298(31)	−24276(20)	46495(52)
O(7′)	18268(14)	−8351(12)	35865(28)
O(8′)	20275(13)	7002(12)	27154(26)
O(9′)	−3309(15)	6449(13)	−6283(37)
O(10′)	−8854(16)	−12098(14)	−25135(32)
O(11′)	−4512(16)	−26145(13)	10398(32)
O(12′)	24414(26)	−23310(16)	71823(38)
O(W1)	−26253(20)	−2185(19)	−53913(44)
O(W2)	23710(20)	−40827(17)	90753(42)

[a]The *y*-coordinate of C(1′) was held constant to define the origin in this direction.

lohexene ring are related to an approximate C_2 symmetry axis passing through the midpoints of the C-2—C-3 and C-5—C-6 bonds, and thus the ring has a half-chair conformation. Hydrogen atoms of the hydroxyl groups and water molecules of crystallization all participate in an extensive hydrogen-bonded arrangement in the solid state. The hydroxyl hydrogen atom involved in the intermolecular O-12′—H12′ . . . N-9 interaction does not lie along the C-8—N-9 line of centers [C-8—N-9 . . . H-12′ = 157 (2)°], and the highly significant departure of the C-7—C-8—N-9 bond angle [172.3(3)°] from strict linearity may be ascribed to the geometrical demands associated with this hydrogen-bonded interaction in the crystal.

Bauhinin did not show any toxicity in mice up to a dose of 300 mg/kg.

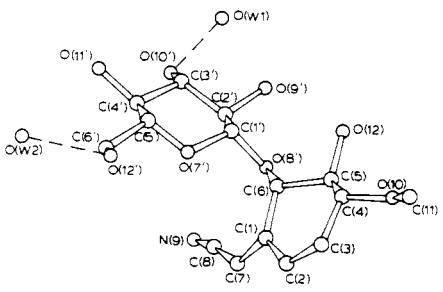

FIGURE 1. Atom numbering scheme and solid-state conformation of bauhinin (**1**) in crystals of the dihydrate; hydrogen atoms have been omitted for clarity. Broken lines denote O-H . . . O hydrogen bonds.

Gallic acid (4) melted at 230-232°. It was identified with an authentic sample by mmp determination, co-tlc, and ir and nmr spectral comparison.

EXPERIMENTAL

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Ir spectra were recorded on a Hitachi 260-30 instrument. Mass spectra were determined on a JEOL JMS-D 100 instrument. Uv spectra were taken on a Perkin-Elmer Lambda-3 spectrometer, and ^{1}H-nmr spectra were measured on JEOL FS-100 MHz and Bruker 250 MHz spectrometers.

ISOLATION OF THE CONSTITUENTS.—Dried and chipped roots (1.5 kg) of *B. championii*,[2] collected at Kuanyinshan, Taipei Prefecture, Taiwan, were extracted successively with boiling *n*-hexane and EtOH. The water-soluble portion of the EtOH extract was chromatographed on an active charcoal (500 g) column, eluted successively with H_2O (10 liters), 10% EtOH (10 liters), 20% EtOH (10 liters), and 40% EtOH (10 liters).

BAUHININ (**1**).—The 40%-EtOH eluate was concentrated, and the residue was recrystallized from H_2O-Me_2CO to give crystals of the dihydrate as colorless prisms: mp 213-214°; uv λ max (MeOH) 256 nm; ir υ max (KBr) 3450-3250, 3050, 2950, 2850, 2220, 1630, 1610, 1410, 1230, 1160-1000(m), 930, 900, 860, and 660 cm^{-1}; ms *m/z* (%) 343(1), 312(1), 282(2), 210(15), 181(30), 163(100), 148(30), 132(25), 104(15), 85(20), and 93(35).

ACETYLATION OF **1**.—Compound **1** was treated with Ac_2O-pyridine at room temperature for 15 h after which H_2O was added and the mixture stirred for 30 min. The solid which separated was collected and recrystallized from MeOH to render bauhinin pentaacetate (**2**): mp 168-170°; ir υ max (KBr) 2955, 2940, 2220, 1750, 1620, 1435, 1240-1210(m), and 1060-1030(m) cm^{-1}; ^{1}H nmr (250 MHz, $CDCl_3$) δ 6.22 (1H, dd, $J_{2,3}$=11 and $J_{2,4}$=1 Hz, H-2), 6.09 (1H, dd, $J_{2,3}$=11 and $J_{3,4}$=3 Hz, H-3), 5.37 (1H, s, H-7), 5.34-5.13 (3H, m, H-2′, H-3′, and H-4′), 5.29 (1H, t, $J_{4,5}$=8 and $J_{5,6}$=8 Hz, H-5), 4.84 (1H, d, J=8 Hz, H-1′), 4.63 (1H, dd, $J_{5,6}$=8 and $J_{4,6}$<1 Hz, H-6), 4.39 (1H, dd, J=13 and 4 Hz, H-6′ or H-7′), 4.12 (1H, dd, J=13 and 4 Hz, H-6′ or H-7′), 4.02 (1H, br d, $J_{4,5}$=8 and $J_{3,4}$=3 Hz, H-4), 3.73 (1H, m, H-5′), 3.40 (3H, s, OMe), 2.15, 2.09, 2.04, 2.03, and 2.02 (3H each, s, 5×OAc).

ENZYMATIC HYDROLYSIS OF **1**.—Compound **1** (150 mg) was dissolved in H_2O (20 ml), and β-glucosidase (50 mg) was added. The reaction mixture was incubated at 28° for 5 days after which the solution was extracted with $CHCl_3$. Evaporation of the organic phase yielded bauhinilide (**3**) (12 mg): mp 119-120°; uv λ max (MeOH) 255 nm; ir υ max (KBr) 3400-3200 (br), 2900, 1770, 1740, 1630, 1150(m), 1080, and 1020 cm^{-1}; ms *m/z* (%) 182 (M^+, 100), 153(82), and 125(95); ^{1}H nmr (100 MHz, $CDCl_3$) δ 6.59 (1H, dd, $J_{2,3}$=10 and $J_{2,4}$=2 Hz, H-2), 6.25 (1H, dd, $J_{2,3}$=10 and $J_{3,4}$=2 Hz, H-3), 5.83 (1H, d, $J_{1,6}$=1 Hz, H-7), 4.80 (1H, dd, $J_{5,6}$=8 and $J_{1,6}$=1 Hz, H-6), 4.02 (1H, br d, $J_{4,5}$=8 Hz, H-4), 3.85 (1H, t, $J_{4,5}$=8 and $J_{5,6}$=8 Hz, H-5), and 3.58 (3H, s, OMe).

The aqueous phase was neutralized with $Ba(OH)_2$, and the resulting suspension was filtered and evaporated. The residue was identified as D-glucose by co-tlc ($CHCl_3$-MeOH, 7:3).

ACIDIC HYDROLYSIS OF **1**.—A solution prepared by dissolving compound **1** (100 mg) in 10% HCl was refluxed for 4 h. After cooling, the resulting mixture was extracted with $CHCl_3$. The $CHCl_3$ and aqueous layers contained products identical with those obtained by enzymatic hydrolysis of **1**, viz. bauhinilide and D-glucose, respectively.

GALLIC ACID (**4**).—The 20% EtOH eluate was concentrated, and the residue was recrystallized from H_2O to give **4** (120 mg) as colorless crystals, mp 230-232°.

CRYSTAL DATA.—Bauhinin dihydrate, $C_{15}H_{20}NO_8.2H_2O$, mol wt=378.36, Monoclinic, a=11.729(1), b=14.205(1), c=5.784(1) Å, β=105.92(1)°, V=926.7 Å^3, Z=2, $D_{calc.}$=1.356 g cm^{-3}, μ (cu-$K\alpha$ radiation, graphite monochromator; λ=1.5418 Å)=9.4 cm^{-1}. Space group $P2_1$ (C_2^2) from systematic absences, $0k0$ when $k=2n$, and **1** is chiral. Sample dimensions 0.14×0.20×0.70 mm.

CRYSTALLOGRAPHIC MEASUREMENTS.—Preliminary unit-cell parameters and space group information were obtained from oscillation, Weissenberg, and precession photographs. Intensity data were recorded on an Enraf-Nonius CAD-4 automated diffractometer (ω-2θ scans, $\theta_{max.}$=67°). From a total of 1714 independent measurements, those 1659 reflections with $I>2.0\sigma(I)$ were retained for the structure analysis and corrected for the usual Lorentz and polarization effects. Refined unit-cell parameters were derived by least-squares treatment of the diffractometer setting angles for 25 high order reflections widely separated in reciprocal space.

[2]A voucher specimen is available for inspection at the Brion Research Institute of Taiwan.

STRUCTURE ANALYSIS.—The crystal structure was solved by direct methods (7). All nonhydrogen atoms were located in an *E*-map. Hydrogen atoms were all found in a difference Fourier synthesis evaluated at a late stage in the analysis. Full-matrix least-squares adjustment of atomic positional and thermal (anisotropic C, N, O; isotropic H) parameters converged to $R=0.042$.[1] Final atomic positional parameters are in Table 1.[3] A view of the asymmetric crystal unit, with the atom numbering scheme, is provided in Figure 1.

Neutral atoms scattering factors used in the structure-factor calculations were taken from Chu and Jeffrey (8). In the least-squares iterations, $\Sigma w\Delta^2$ ($\Delta=||F_o|-|F_c||$) was minimized, with weights, w, assigned according to the scheme: $\sqrt{w}=1$ for $|F_o|<8.6$, and $\sqrt{w}=8.6/|F_o|$ for $|F_o|>8.6$.

ACKNOWLEDGMENTS

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[3]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.