Indicanine A, a New 3-Phenylcoumarin from Root Bark of *Erythrina indica*[1]

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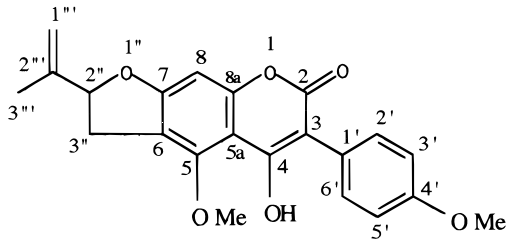
A new 3-phenylcoumarin, indicanine A (**1**), has been isolated from the root bark of the African medicinal plant *Erythrina indica*, together with three known compounds, robustic acid (**2**), daidzein, and 8-prenyldaidzein. The structure of the new compound was characterized, as 4-hydroxy-5-methoxy-3-(4′-methoxyphenyl)-2″-(1-methylethenyl)dihydrofurano[4″,5″:6,7]coumarin by means of extensive spectroscopic analyses. The compounds were found to be devoid of *in vitro* antibacterial activity.

Plants species belonging to genus *Erythrina* (Papilionaceae) are well-known for elaborating, from seeds and leaves, alkaloids possessing cardiovascular effects[2] and, from stem and root barks, isoflavonoids (pterocarpans, isoflavones, isoflavanones)[3−5] and flavonoids (chalcones, flavanones),[6,7] of which some exhibit antibacterial and antifungal activity,[7,8] as well as inhibited platelet aggregation.[8]

As part of our continuing investigation on the phenolic metabolites from *Erythrina* species found in Cameroon and elsewere, we have examined the chemical constituents of the root bark of *E. indica*, a plant used extensively in African folk medicine for the treatment of several diseases, including microbial infections.[9] In this paper, we report the isolation and structure elucidation of a new 3-phenylcoumarin, designated indicanine A, along with three known co-occurring compounds, the phenylcoumarin robustic acid (**2**), and the isoflavones daidzein and 8-prenyldaidzein.



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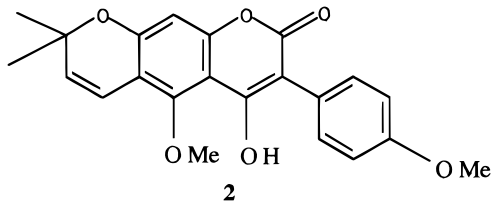

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The dried and ground root bark of *E. indica* was successively extracted at room temperature with a mixture of CH_2Cl_2−MeOH (1:1) and the extract concentrated to dryness. The residue showed antimicrobial activity against *Staphylococcus aureus* and *Mycobacterium smegmatis* at <1000 μg/mL in the agar dilution-streak assay.[10] This residue, on repeated column chromatographic separations over Si gel, afforded pure compounds, including indicanine A (**1**) and the three known compounds, robustic acid (**2**),[11,12] daidzein,[13] and 8-prenyldaidzein,[14] which were identified by comparison with reported spectoscopic data.

Compound **1**, mp 175−177 °C, was obtained as an optically active pale yellow amorphous solid, with molecular formula $C_{22}H_{20}O_6$, as established by mass spectrometry. The broad-band decoupling ^{13}C NMR spectrum of **1** showed 20 carbon signals. The analysis of this spectrum with the aid of J_{Mod} and DEPT techniques unequivocally indicated the presence of three methyl groups, two methylene carbons, and six methine groups. Thus, there were 11 quaternary carbons, all sp^2. The IR spectrum showed bands attributable to hydroxyl (3267 cm^{-1}), conjugated carbonyl (1645 cm^{-1}), and benzene ring (1610 and 1520 cm^{-1}). The ^1H NMR signal at δ 10.0 ppm, ^{13}C NMR signal at δ 162.7 ppm (C-2), and UV absorption bands at 218, 270, 282 sh, 291, 305 sh, and 351 nm were all typical of a 3-phenylcoumarin skeleton.[15,16] These data agreed closely with those reported for robustic acid (**2**).[11,12] Addition of NaOAc did not cause a bathochromic shift, thus suggesting that there is no free phenolic group at C-7. In the ^1H NMR spectrum, the D_2O exchangeable signal at δ 10.0 ppm is characteristic of 4-OH resonance.[11,12] Also, the ^1H NMR spectrum of **1** shows an AA′BB′ spin system of four phenyl protons at δ 7.42 ppm (2H, d, J = 8.8 Hz, H-2′ and H-6′) and δ 6.93 ppm (2H, d, J = 8.8 Hz, H-3′ and H-5′), indicating the presence of a *para*-substituted ring B and a 1H singlet at δ 6.58 ppm due either to H-6 or H-8 of ring A. Furthermore, the set of signals consisting of two 1H doublets at δ 5.0 ppm (1H, J = 1.0 Hz) and δ 4.95 ppm (1H, d, J = 1.0 Hz), due to two geminal olefinic protons and a 3H signal at δ 1.77 ppm establish the presence of an isopropenyl substituent. The presence of a dihydrofuran unit was supported by two 3H singlets at δ 4.08 and 3.80 ppm due to two methoxyl groups and two sets of two double doublets at δ 3.58 (1H, dd, J = 8.8 and 15.3 Hz) and δ 3.23 ppm (1H, dd, J = 7.6 and 15.8 Hz) for the two diastereotopic protons at C-3″ and 1H triplet at δ 5.28 ppm (1H, t, J = 8.4 Hz, H-2″). On the basis of these data, compound **1** must be a 4-hydroxy-3-phenyl coumarin substituted in ring A with one methoxyl group at C-5 position and an isopropenyldihydrofuran moiety. This was confirmed by EIMS of indicatine A (**1**), which undergoes RDA fragmen-

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tation via its keto tautomer to give two important ion fragments at *m*/*z* 233 and 148. The first ion fragment at *m*/*z* 233, clearly showed compound **1** possesses one methoxyl and an isopropenyldihydrofuran moiety on ring A, while the second ion fragment at *m*/*z* 148, indicated the second methoxyl group to be located on ring B at C-4′ position. Therefore, it remained to be established unambiguously whether the fusion of the isopropenyl dihydrofuran moiety on ring A is linear or angular. This was deduced from NOE difference experiments, which showed no enhancement of the 1H aromatic signal at δ 6.58 ppm (corresponding either to H-6 or H-8) but enhancement of the signal at δ 10.0 ppm (4-OH), when the signal at δ 4.08 ppm (5-OMe) was irradiated. This finding clearly indicated that the single A-ring aromatic proton at δ 6.58 ppm was located at C-8. Thus, the isopropenyl dihydrofuran unit was fused in a linear manner on ring A. From the above spectroscopic studies, compound **1** was characterized as 4-hydroxy-5-methoxy-3-(4′-hydroxyphenyl)-2″-(1-methylethenyl)dihydrofuran-[4″,5″:6,7]coumarin. This compound, which appears to be novel, has been given the trivial name indicanine A. We were not able to establish the absolute configuration at the C-2″ stereocenter.

All the isolated compounds were tested in vitro for their antimicrobial activities against microorganisms, *S. aureus* 209P, *M. smegmatis* ATCC 607, and *Escherichia coli* RL65 using an agar dilution-streak method.[10] None of the compounds showed any significant activity.

Experimental Section

General Experimental Procedures. All melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 727B spectrometer in KBr disks. UV spectra were obtained on a Beckman model 25 spectrophotometer. EIMS (ionization voltage, 70 eV) were measured with LKB9000S and Nermag/sidar U 3:1 spectometers. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 2000 and on a Bruker spectrometers equipped with a 5-mm ^{1}H and ^{13}C probe operating at 300 and 75 MHz, respectively, with TMS as internal standard. DEPT and J_{Mod} were measured with the usual pulse sequence, and data processing was obtained with standard software.

Plant Material. Root bark of *E. indica* was collected in June 1998, at Ibadan, Nigeria. A voucher specimen documenting the collection is on deposit at the National Herbarium, Yaounde, Cameroon.

Extraction and Isolation. Air-dried, powdered root bark of *E. indica* (6 kg) was extracted with a mixture of CH_2Cl_2−MeOH (1:1) and concentrated to dryness on a rotary evaporator under reduced pressure to afford a viscous mass of CH_2Cl_2−MeOH (1:1) extract (200 g). This material was subjected to column chromatography on Si gel (70−230 mesh, ASTM; Merck) packed in *n*-hexane and eluted with *n*-hexane−EtOAc mixture. In all, 200 fractions (ca. 250 mL each) were collected and combined on the basis of TLC analysis, leading to five main series (A−E). Fractions 1−50, eluted with a mixture of hexane−EtOAc (9:1), gave series A, from which robustic acid (**2**) (4 g) crystallized. Fractions 101−120, eluted with hexane−EtOAc (3:2), gave series C, which was further subjected to repeated column chromatography over Si gel eluted with a mixture of hexane−EtOAc (7:3) to yield indicanine A (**1**) (70 mg). Series D, resulting from the combination of fractions 121−181 eluted with a mixture of hexane−EtOAc (1:1), was rechromatographed with Si gel column chromatography, eluting with hexane−EtOAc (3:2) to afford daidzein (60 mg) and 8-prenyldaidzein (100 mg).

Indicanine A (1): pale-yellow powder, mp 175−177 °C, yield 0.0012% $[\alpha]_D$ −46° (*c* 1.99, MeOH); UV (MeOH) λ_{max} (log ϵ) 218 (4.54), 270 (3.48), 282 sh (3.86), 291 (4.23), 305 sh (4.17), 351 (4.63) nm; IR ν_{max} (KBr) 3267, 1645, 1610, 1520, 1200, 1100 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.42 ppm (1H, d, J = 8.8 Hz, H-2′ and H-6′), 6.93 (2H, d, J = 8.8 Hz, H-3′and H-5′), 6.58 (1H, s, H-8), 5.28 (1H, t, J = 8.8 Hz, H-2″), 5.0 (1H, d, J = 1.0 Hz, =CH), 4.95 (1H, d, J = 1.0 Hz, =CH), 4.08 (3H, s, 5-OMe), 3.80 (3H, s, 4′-OMe), 3.58 (1H, dd, J = 8.8 and 15.5 Hz, H-3″), 3.23 (1H, dd, J = 7.6 and 15.8 Hz, H-3″), δ 1.77 (3H, s, CH_3−C=C); ^{13}C NMR (75 MHz) δ 164.1 (s, C-4), 162.7 (s, C-2), 161.1 (s, C-5), 158 (s, C-7), 155.1 (s, C-4′), 152.4 (s, C-8a), 142.4 (s, C-2‴), 131.7 (d, C-2′ and C-6′), 123.6 (s, C-1′), 113.5 (t, C-1‴), 113.0 (d, C-3′ and C-5′), 111.0 (s, C-6), 100.0 (s, C-5a), 86.1 (d, C-2″), 60.5 (q, 5-OMe), 55.2 (q, 4′-OMe), 33.2 (t, C-3″), 17.1 (q, 3‴-Me); EIMS *m*/*z* [M]$^+$ 380 (96), 365 (33), 337 (19), 233 (100), 217 (33), 190 (44), 175 (28), 148 (98), 135 (33), 120 (41), 91 (30), 69 (43), 41 (21), 39 (16); DCI/NH_3 [M+1]$^+$ 381; HREIMS *m*/*z* [M$^+$] 380.1262 (calcd for $C_{22}H_{20}O_6$, 380.1264).

Robustic acid (2): white solid, yield 0.066%, mp 212° (lit.[12] 210°C); HEIMS *m*/*z* [M$^+$] 380.1261 (calcd for $C_{22}H_{20}O_6$, 380.1260); UV, IR, ^{1}H and ^{13}C NMR were in agreement with the published data.[11,12]

Daidzein: amorphous powder, yield 0.0001%, mp 198−200 °C; HREIMS *m*/*z* 254.0577 (calcd for $C_{15}H_{10}O_4$, 254.O579); IR, ^{1}H and ^{13}C NMR spectra data were in agreement with literature values.[13]

8-Prenyldaidzein: white amorphous solid, yield 0.0001%, mp 198 °C (lit.[14] 196−198 °C); HREIMS *m*/*z* 322.1206 (calcd for $C_{20}H_{18}O_4$, 322.1205); IR, ^{1}H and ^{13}C NMR spectral data matched well with those published in the literature.[14]

Antimicrobial Activity Screening. Extract and purified active compounds were tested at 1 mg/mL against *S. aureus* 209P, *M. smegmatis* ATCC 607, and *E. coli* RL65. The three strains of bacteria were cultured in Mueller−Hinton agar medium at 37 °C. After one day, their growth was assessed visually. The lowest concentration of the test compounds in which no visible growth occurred was defined as the minimum inhibitory concentration.

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