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Indicanines B and C, two isoflavonoid derivatives from the root bark of *Erythrina indica*☆

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Abstract

In addition to two known compounds, 5,4′-di-*O*-methylalpinumisoflavone and cajanin, a new 3-phenylcoumarin metabolite, named indicanine B, and a new isoflavone derivative, named indicanine C, were isolated from the root bark of *Erythrina indica*. By means of spectroscopic analysis, the structures of the new compounds were characterized as 4-hydroxy-3-(4′-hydroxyphenyl)-5-methoxy-2″,2″-dimethylpyrano [5″,6″:6,7] coumarin and 4′-hydroxy-5-methoxy-2″,2″-dimethylpyrano [5″,6″:6,7] isoflavone, respectively. The ^{13}C-NMR data of cajanin and the in vitro antimicrobial spectrum and potencies of the isolated compounds are also reported. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Erythrina indica*; Papilionaceae; Leguminosae; Root bark; Indicanines B and C; 5,4′-di-*O*-methylalpinumisoflavone; Cajanin; Phenylcoumarin; Isoflavones; Antibacterial activity

1. Introduction

There has been a recent increase in the research effort on the non-alkaloidal secondary metabolites of the genus *Erythrina* (Drake, Gerlach, Gollapudi, Mitscher, Veliz & Ward, 1988; Fomum, Koch, Seguin, Tillequin & Wandji, 1994; Berggendorff, Meyer, Nkengfack, Olov & Vouffo, 1994; Fomum, Kouam, Nkengfack, Olov, Vardamides & Vouffo, 1997). Our interest was stimulated when in our hands and other laboratories, several phenolic metabolites, such as pterocarpans, isoflavones, flavanones and chalcones were isolated from the root and bark of *Erythrina* species, of which some displayed antimicrobial activity and inhibited platelet aggregation (Fomum et al., 1997; Chuo, Kamat, Kubo & Nakanishi, 1981; Gollapudi, Keshavarz-Shorkri, Mitscher & Okwute, 1988).

Erythrina indica (Leguminosae) is an important medicinal plant used in Cameroonian folk medicine for the treatment of several diseases including trachoma, elephantiasis and microbial infections (Ayensu, 1978; Dalziel, 1937). In a previous report, we have described the isolation and structure elucidation of a new antimicrobial 3-phenylcoumarin, indicanine A, along with the known compounds, robustic acid, daidzein and 8-prenyl daidzein from the root bark of *Erythrina indica* (Azebaze et al., 2000).

In the present paper, we wish to describe the structure elucidation of four compounds isolated from the root bark of this plant, i.e. a new 3-phenylcoumarin named indicanine B (**1**), a new isoflavone named indicanine C (**2**) and two known isoflavones, 5,4′-di-*O*-methylalpinumisoflavone (**3**) and cajanin (**4**).

☆ Part 36 in the series "Erythrina studies"

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2. Results and discussion

Dried and ground root bark of *Erythrina indica* was successively extracted with a mixture of dichloromethane–MeOH (1:1) and methanol. The dichloromethane–MeOH (1:1) extract was concentrated to dryness. This extract, when assayed for antimicrobial activity, gave a wide inhibition zone against the Gram positive bacterium, *Staphylococus aureus* (209P) and *Mycobacterium smegmatis* (ATCC 607). Extensive column chromatography of this residue over silica gel, afforded pure indicanine B (**1**) and C (**2**) together with the two known compounds, (**3**) and (**4**).

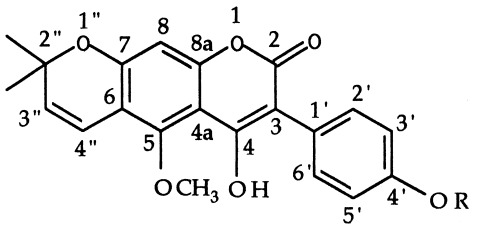
Compounds (**3**) and (**4**) were characterized as isoflavones from their spectral data and identified as 5,4′-di-*O*-methylalpinumisoflavone (**3**) and cajanin (**4**) by comparison of their spectral data with literature values (Bettolo, Lwande, Monache Olivares, 1982; Khalid & Waterman, 1983; Jackson, Owen & Scheinmann, 1971; Bilyard, Cooksey, Dahiya, Garratt & Strange, 1984; Ingham & Markham, 1980). The ^{13}C-NMR spectral data (see Section 3) of cajanin (**4**) are reported here for the first time. The assignments were made by comparison with data published for related compounds (Agrawal & Bansal, 1989), as well as the multiplicities obtained from J_{Mod} and DEPT spectra.

Compound (**1**), indicanine B, was obtained as colourless amorphous powder and reacted positively to the $FeCl_3$ reagent. Its high-resolution EI-mass spectrum displayed a molecular ion [M^+] at m/z 366.1102 in agreement with the empirical formula $C_{21}H_{18}O_6$ (366.1103) corresponding to 13° of unsaturation. The IR spectrum exhibited absorption bands for free hydroxyl (3400) chelated hydroxyl (3238), conjugated carbonyl (1683), olefine (1631, 1514) and ether (1280, 1140 cm^{-1}) functionalities. The UV spectrum of indicanine B (**1**) showed absorption bands at λ_{max} 234, 258 and 339 nm, typical of a compound having a 3-arylcoumarin skeleton (Bettolo et al., 1982; Khalid & Waterman, 1983). In fact, this UV spectrum as well as ^{1}H- and ^{13}C-NMR spectra were very similar to those of robustic acid (**1a**), a 3-phenyl coumarin derivative also isolated in large amount from the same plant. The comparison of spectral data of these two compounds revealed the presence of the same type of substituents in both compounds, i.e. a gem-dimethyl pyran moiety, a *para* disubstituted aromatic ring and methoxyl groups with the exception that there was only one methoxy group in (**1**), compared to two methoxy groups in robustic acid (**1a**). This methoxyl group, whose signal resonance appeared as a 3H singlet at δ3.88 ppm in ^{1}H-NMR and δ64.2 ppm in ^{13}C-NMR, was located at the C-5 position. The placement of the substituents on either ring A or B of indicanine B (**1**) was confirmed by the fragmentation patterns observed in the mass spectrum. The fragment ions at m/z 217 and 134 can be attributed to the normal RDA cleavage of the fragment with m/z 351 [M-15]$^+$. The ion peak at m/z 134 clearly corroborated the presence of *para* disubstituted aromatic B ring bearing a hydroxy phenolic group at C-4′ position, while the ion peak m/z at 217 support the presence of the 2,2-dimethylpyran moiety on ring A. It was established unambiguously by the NOE difference experiment that this dimethylpyran unit was fused on ring A in a linear manner. NOE showed no signal enhancement of the single aromatic proton of ring A at δ6.65 ppm when the methoxy protons were irradiated, suggesting that this proton is at C-8 position. Rather, an enhancement (29%) of the signal due to H-4″ olefinic proton of pyran moiety at δ6.63 ppm was observed indicating the close spatial proximity of this proton with respect to the methoxyl group. On the basis of the above spectroscopic studies, indicanine B (**1**) was given as 4-hydroxy-3-(4′-hydroxyphenyl)-5-methoxy-2″,2″-dimethylpyrano [5″,6″: 6,7] coumarin.

The second compound (**2**), named indicanine C, was obtained as a pale amorphous powder, mp. 195°C and reacted positively to $FeCl_3$ reagent. Its molecular formula, $C_{21}H_{18}O_5$, was deduced from its high resolution EI-mass spectrum (M^+, m/z 350.1152, calculated for $C_{21}H_{18}O_5$, 350.1154). Its IR spectrum showed absorption bands for free hydroxyl (3325), conjugated carbonyl (1647) olefin (1602) and ether (1272, 1235 cm^{-1}) functionalities. The UV, ^{1}H- and ^{13}C-NMR spectral data (see Section 3) of indicanine C were very similar to those of 5,4′-di-*O*-dimethylalpinumisoflavone (**3**) (Bettolo et al., 1982; Khalid & Waterman, 1983; Jackson et al., 1971), with the presence of a 1H singlet at δ7.76 characteristic of the H-2 proton of an isoflavone and signals for a gem-dimethylpyran unit and *para* disubstituted aromatic ring B, suggesting an analogous substitution pattern. The main difference between the two compounds arose from the fact that in compound (**3**) there were two methoxyl groups, whereas, there was only one methoxy group in indicanine C (**2**). The appearance of its signal in ^{1}H- and ^{13}C-NMR as a 3H singlet at δ_H 3.89 and δ_C 62.9, led us to locate this methoxyl substituent at C-5 position. This was readily supported by the EI-mass spectrum which displayed RDA ion peaks, respectively, at m/z 217 and 118, thus confirming 5- rather than 4′-*O*-methylation. The linear fusion of the dimethylpyran unit on ring A was corroborated by the NOE difference spectroscopy, which showed an enhancement of the signal due to H-4″ proton (δ6.72 ppm) of the pyran moiety, when the methoxyl protons were irradiated. From the above spectroscopic studies, indicanine C (**2**), also called 5-*O*-methylalpinumisoflavone, was deduced to be 4′-hydroxy-5-methoxy-2″,2″-dimethylpyrano [5″,6″: 6,7] isoflavone.

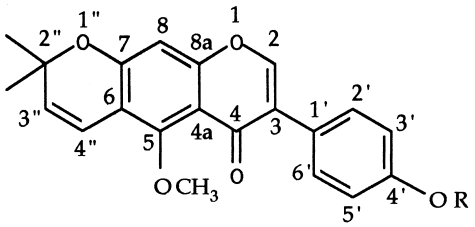
Indicanine B (**1**), indicanine C (**2**), 5,4′-di-*O*-methy-

lalpinumisoflavone (**3**) and cajanin (**4**) were tested in vitro for their antimicrobial activities using an agar streak-dilution technique (Mitscher, 1977). Only indicanine B (**1**) was active against *S. aureus* and *M. smegmatis* (Table 1). Other compounds were found to be inactive at lower concentration (during our experiment).



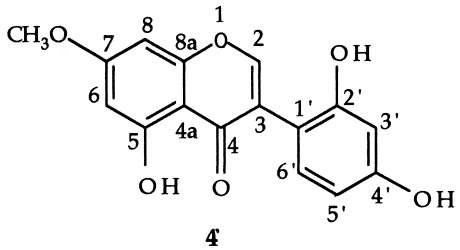
indicanine B 1: R= H

robustic acid 1a: R= Me



2 : R= H, **indicanine C**

3 : R= Me, 5,4'-di-O-methylalpinumisoflavone



4

cajanin

Table 1
Antimicrobial activity of isolated components in vitro

Microorganisms[a] tested (MIC, μgml^{-1})			
Sample	1	2	3
E. indica (root bark)	1000	> 1000	500
Phenolic fractions	19.5	> 1000	17.5
Indicanine B (**1**)	9.7	> 1000	18.5
Indicanine C (**2**)	> 300	> 1000	> 150
Dimethylalpinumisoflavone (**3**)	> 1000	> 1000	> 450
Cajanin (**4**)	> 1000	> 1000	> 400
Streptomycin	5.5	5.0	1.7

[a] Microorganisms: 1 = *Staphylococcus aureus* 209P; 2 = *Escherichia coli* RL265; 3 = *Mycobacterium smegmatis* ATCC607.

3. Experimental

3.1. General experimental procedures

All melting points were determined on Buchi 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. EI-MS (ionization voltage, 70 eV) were measured with a Finnigan–Mat 8200 spectrometer. ^{1}H- and ^{13}C-NMR spectra were taken on a Varian Gemini 2000 and a Bruker spectrometer equipped with a 5-mm ^{1}H- and ^{13}C probe operating at 300 and 75 MHz. DEPT and J_{Mod} were measured with the usual pulse sequence.

3.2. 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, Cameroun.

3.3. Extraction and isolation

Air dried, powdered root bark of *E. indica* (6 kg) was successively extracted with a mixture of CH_2Cl_2–MeOH (1:1) and methanol. The CH_2Cl_2–MeOH (1:1) was concentrated to dryness on a rotary evaporator under 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.

A total of 200 fractions of ca. 250 ml each were collected and combined on the basis of TLC analysis leading to five main series (A–E). Fractions 50–100, eluted with of a mixture of hexane–EtOAc (9.1), gave series B from which 5,4′-di-*O*-methylalpinumisoflavone (**3**) (4.8 g) crystallized. The filtrate was concentrated to dryness to give a thick residue. This residue, on repeated column chromatography over Si gel eluted with hexane–EtOAc mixtures of increasing polarity, yielded indicanine C (**2**) (23 mg). Fractions 200–250, eluted with hexane–EtOAc (1:1), gave the series E. This series was further subjected to repeated column chromatography over Si gel eluted with a mixture of hexane–EtOAc (7:3) to yield indicanine B (**1**) (85 mg) and cajanin (**4**) (48 mg).

3.3.1. Indicanine B (*1*)

Colourless amorphous powder, mp 200–201°C; HRMS m/z: 366.1102 (calculated for $C_{21}H_{18}O_6$: 366.1103); UV (MeOH) λ_{max} (log ε) 234 (4.23), 258 (3.48), 339 nm (3.86); IR ν_{max} (KBr) 3400, 3238, 1683, 1631, 1514, 1405, 1331, 1280, 1140 and 1100 cm^{-1}; ^{1}H-

NMR (DMSO, 300 MHz) 10.0 (1H, *s*, exchangeable D_2O, 4-OH), 9.42 (1H, *s*, exchangeable D_2O, 4′-OH), 7.21 (2H, *d*, J = 8.8 Hz, H-2′ and H-6′), 6.78 (2H, *d*, J = 8.8 Hz, H-3′ and H-5′), 6.65 (1H, *s*, H-8), 6.63 (1H, *d*, J = 10.0 Hz, H-4″), 5.91 (1H, *d*, J = 10.0 Hz, H-3″), 3.88 (3H, *s*, OMe-5), 1.42 ppm (6H, *s*, 2″-Me_2); ^{13}C-NMR (75 MHz) δ161.5 (*s*, C-4), 160.3 (*s*, C-2), 156.4 (*s*, C-7), 156.3 (*s*, C-5), 153.4 (*s*, C-4′), 152.7 (*s*, C-8a), 132.0 (*d*, C-2′ and C-6′), 131.2 (*d*, C-3″), 122.0 (*s*, C-1′), 115.1 (*d*, C-4″), 114.5 (*d*, C-3′ and C-5′), 110.0 (*s*, C-6), 103.3 (*s*, C-3), 102.5 (*s*, C-4a), 100.2 (*d*, C-8), 77.5 (*s*, C-2″), 64.2 (*q*, 5-OMe), 27.6 (*q*, 2″-Me_2); EIMS m/z [M^+] 366 (96), 351 (100), 217 (70), 188 (34), 134 (64).

3.3.2. Indicanine C (**2**)

Amorphous powder, mp 195°C. HRMS m/z 350.1152 (calculated for $C_{21}H_{18}O_5$: 350.1154); UV (MeOH) λ_{max} (log ε) 288 (4.78) nm; IR ν_{max} (KBr) 3325, 2981, 1654, 1646, 1602, 1272, 1235, 1067 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): δ7.76 (1H, *s*, H-2), 7.26 (2H, *d*, J = 8.7 Hz, H-2′, H-6′), 7.19 (1H, *br*, exchangeable with D_2O, 4′-OH), 6.82 (2H, *d*, J = 8.7 Hz, H-3′ and H-5′), 6.72 (1H, *d*, J = 10 Hz, H-4″), 6.60 (1H, *s*, H-8), 5.72 (1H, *d*, J = 10 Hz, H-3″), 3.89 (3H, *s*, 5-OCH_3) and 1.47 [6H, *s*, $(CH_3)_2$ C]; ^{13}C-NMR (75 MHz, $CDCl_3$): δ175.8 (*s*, C-4), 158.7(*s*, C-7), 158.2 (*s*, C-5), 156.4 (*s*, C-4′), 155.7 (*s*, C-8a), 150.7 (*d*, C-2), 130.8 (*d*, C-3″), 130.4 (*d*, C-2′ and C-6′), 125.9 (*s*, C-3), 123.4 (*s*, C-1′), 116.0 (*d*, C-4″), 115.8 (*d*, C-3′ and C-5′), 113.4 (*s*, C-6), 113.1 (*s*, C-4a), 100.7 (*d*, C-8), 77.8 (*s*, C-2″), 62.9 (*q*, 5-OMe) and 28.3 ppm (*q*, Me_2-2″); EIMS m/z (rel. int.) [M^+] 350 (78), 335 (100), 306 (18), 217 (45), 202 (23), 168 (57), 118 (49).

3.3.3. 5,4′-di-O-methylalpinumisoflavone (**3**)

Colourless needles, mp 121°C (lit. Khalid & Waterman, 1983; Jackson et al., 1971 119–121°C). HRMS m/z 364.1306 (calculated for $C_{22}H_{20}O_5$: 364.1310); UV, IR, ^{1}H- and ^{13}C-NMR and EIMS in closed agreement with published data (Khalid & Waterman, 1983; Jackson et al., 1971).

3.3.4. Cajanin (**4**)

Amorphous powder, mp 208–210°C. HRMS m/z 300.0632 (calculated for $C_{16}H_{12}O_6$: 300.0634); UV and IR spectral data, matched well with the published value (Bilyard et al., 1984); ^{1}H-NMR (300 MHz, DMSO): 12.98 (1H, *s*, exchangeable with D_2O, OH-5), 9.39 (1H, *s*, exchangeable with D_2O, 2′-OH), 9.31 (1H, *s*, exchangeable with D_2O, 4′-OH), 8.22 (1H, *s*, H-2), 6.96 (1H, *d*, J = 8.3 Hz, H-6′), 6.63 (1H, *d*, J = 2.3 Hz, H-3′), 6.36 (1H, *dd*, J = 2.3 and 8.3 Hz, H-5′), 6.28 (1H, *d*, J = 2.2 Hz, H-6), 6.24 (1H, *d*, J = 2.2 Hz, H-8), 3.85 (3H, *s*, 7-OMe); ^{13}C-NMR (75 MHz, DMSO): 180.6 (*s*, C-4), 165.1 (*s*, C-7), 161.6 (*s*, C-5), 158.6 (*s*, C-8a), 157.5 (*s*, C-4′), 156.4 (*s*, C-2′), 155.6 (*s*, C-2), 132.2 (*d*, C-6′), 120.6 (*s*, C-3), 108.4 (*s*, C-1′), 106.2 (*d*, C-5′), 105.4 (*s*, C-4a), 102.6 (*d*, C-3′), 97.9 (*d*, C-6), 92.3 (*d*, C-8), 56.1 (q, 7-OMe).

3.4. Antimicrobial activity screening

The Minimal Inhibition Concentration (MIC) was defined as the lowest concentration of antimicrobial agents in the agar medium resulting in complete inhibition of visible growth. The MIC of the tested compounds were determined by the agar-streak dilution technique against representative Gram-positive and negative organisms. The results obtained are given in Table 1.

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