PRENYLATED ISOFLAVONOIDS FROM *ERYTHRINA SENSEGALENSIS**

Jean Wandji, S. Suh Awanchiri, Z. Tanee Fomum,† François Tillequin‡ and S. Michel-Daniwicz‡

Department of Organic Chemistry, University of Yaounde, P.O. Box 812, Yaounde, Cameroon; ‡Département de Pharmacognosie, Université de René Descartes, U.R.A. au C.N.R.S. N° 1310, Faculté des Sciences, Pharamaceutiques et Biologiques, 4 Avenue de l' Observatoire F, 75006 Paris, France

Abstract—Two novel prenylated isoflavonoids, 3,5,4'-trihydroxy-8-(γ,γ-dimethylallyl)-2'', 2''-dimethylpyrano [5'',6'':6,7]coumaronochromone and 5,2',4'-trihydroxy-8-(γ,γ-dimethylallyl)furano [4'',5'':6,7] isoflavone, have been isolated from the stem bark of the Cameroonian medicinal plant, *Erythrina senegalensis*, in addition to five known pentacyclic triterpenes, β-amyrin, maniladiol, erythrodiol, oleanolic acid and cornulacic acid. Their structures were determined by spectroscopic methods.

INTRODUCTION

Chemical studies of some Leguminosae species have revealed the co-occurrence of coumaronochromones and isoflavonoid compounds [1–4]. Our on-going chemical studies on the non-alkaloidal components of the Cameroonian medicinal plant *Erythrina senegalensis* have isolated and determined the structures of a number of new isoflavones [5–8]. In continuation of these studies, we now report the isolation and structural elucidation of a new 3-hydroxycoumaronochromone, erysenegalensein J (**1**) and a new furanoisoflavone, erysenegalensein K (**2**).

RESULTS AND DISCUSSION

By repeated column chromatography on silica gel and prep. TLC of a dichloromethane extract of the stem bark of *E. senegalensis*, two novel compounds **1** and **2** were obtained.

Compound **1**, erysenegalensein J, was obtained as a gum and its molecular formula $C_{25}H_{24}O_7$ deduced from the HR mass spectrum showed the exact mass at m/z 436.1817. This was then confirmed by CI- and EI-mass spectra which showed, respectively, ions at m/z 437 [M + H]$^+$ and m/z 436 [M]$^+$. Its IR spectrum showed bands at 3400 (free OH), 3000 (chelated OH) and 1640 cm^{-1} (C = O chelated) suggesting an isoflavonoid or flavonoid skeleton with a free hydroxyl group at the C-5 position and a carbonyl function at the C-4 position [9]. This hypothesis was confirmed by its UV (MeOH) spectrum which exhibited two intense absorption bands at

276 and 322 nm and its ^{1}H NMR spectrum (Table 1) which showed the OH-5 singlet at δ11.60, exchangeable on deuteration. Compound **1** contained one dimethylchromen group which showed two doublets at δ5.51 (1H, d, J = 10.0 Hz, H-3'') and 6.58 (1H, d, J = 10.0 Hz, H-4''), two methyl singlets at δ1.40 and 1.45; the presence of one γ,γ-dimethylallyl group was indicated by singals at δ1.65 (3H, s, Me-*trans*), 1.70 (3H, s, Me-*cis*), 3.22 (2H, d, CH$_2$ allylic) and 5.14 (1H, t, CH =C <). The presence of the two five-carbon units was confirmed by the EI-mass spectrum which showed important ion fragments at m/z 421 [M − Me]$^+$, 287, 271 and 150. The ion fragments at m/z 287 and 150 resulted from retro-Diels–Alder cleavage with hydrogen transfer of the [M]$^+$ (Scheme 1). The first one (m/z 287) confirmed that the two five-carbon units were linked to ring A, notably at the C-6 and C-8 positions since the ^{1}H NMR spectrum did not show a signal at δ5.90–6.10, characteristic of the protons at these positions. However, it remained to establish unambiguously the orientations of the two units. The second ion at m/z 150 [C$_8$H$_6$O$_3$]$^+$ resulted from rings B and C.

Further analysis of the ^{1}H NMR spectrum showed ABX-system signals corresponding to three ring B protons at δ6.48 (d, J = 2.0 Hz, H-3'), 6.45 (dd, J = 2.0, 8.0 Hz, H-5') and 7.20 (d, J = 8.0 Hz, H-6'). The large singlet at δ3.92, assigned to the hydroxyl group at the C-3 position, exchangeable on deuteration, and the singlet at δ6.28 corresponding to H-2, might result from the addition of water to the double bond C-2/C-3 in ring C of the coumaronochromone skeleton. Assignments were confirmed by the ^{13}C NMR spectrum which showed three ring C carbon signals at δ109.7 (C-2), 77.8 (C-3) and 192.0 (C-4) (Table 1); the two ring B *meta*-oxygenated carbon signals at δ161.0 (C-2') and 161.3 (C-4') were in agreement with the literature [10].

---
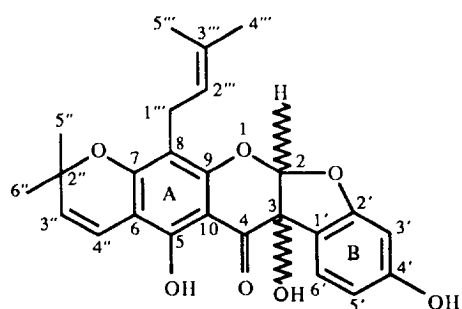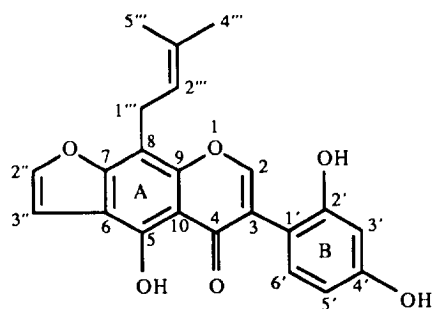
(1)   (2)
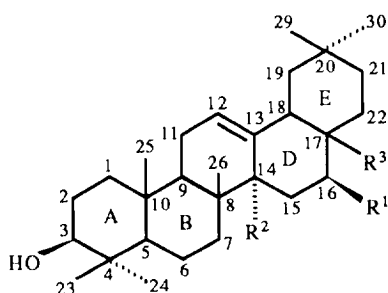

(3) $R^1 = H$, $R^2 = R^3 = CH_3$
(4) $R^1 = OH$, $R^2 = R^3 = CH_3$
(5) $R^1 = H$, $R^2 = CH_3$, $R^3 = CH_2OH$
(6) $R^1 = H$, $R^2 = CH_3$, $R^3 = COOH$
(7) $R^1 = H$, $R^2 = COOH$, $R^3 = CH_3$

Table 1. ^1H and ^{13}C NMR spectral data for 1 and 2 (in $CDCl_3$)

Atom	1 ^{13}C	1 ^1H J (Hz)	2 ^{13}C	2 ^1H J (Hz)
2	109.7	6.28 1H, s	154.4	8.15 1H, s
3	77.8	—	123.9	—
4	192.0	—	183.9	—
5	155.8	—	159.2	—
6	99.5	—	107.8	—
7	156.5	—	159.1	—
8	109.4	—	107.0	—
9	161.1	—	157.1	—
10	103.6	—	104.2	—
1'	117.7	—	111.2	—
2'	161.0	—	152.7	—
3'	110.0	6.48 1H, d (2.0)	106.0	6.60 1H, d, (2.0)
4'	161.3	—	154.8	—
5'	98.9	6.45 1H, dd (8.0, 2.0)	108.7	6.50 1H, dd (8.0, 2.0)
6'	125.2	7.20 1H, d (8.0)	130.9	7.05 1H, d (8.0)
2"	78.7	—	144.4	7.64 1H, d (2.0)
3"	126.3	5.51 1H, d (10.0)	103.7	7.00 1H, d (2.0)
4"	115.1	6.58 1H, d (10.0)	—	—
5"	28.3	1.40ᵃ 3H, s	—	—
6"	28.3	1.45ᵃ, 3H, s	—	—
1'''	21.1	3.22 2H, d (7.0)	21.9	3.64 2H, d (7.0)
2'''	121.8	5.14 1H, t (7.0)	120.8	5.36 1H, t (7.0)
3'''	131.6	—	132.2	—
4'''	25.7	1.65 3H, s	22.5	1.72 3H, s
5'''	17.7	1.70 3H, s	17.7	1.88 3H, s
OH-3*	—	3.92 1H, s	—	—
OH-5*	—	11.60 1H, s	—	12.70 1H, s

*Exchanged on deuteration.
ᵃValues may be reversed.
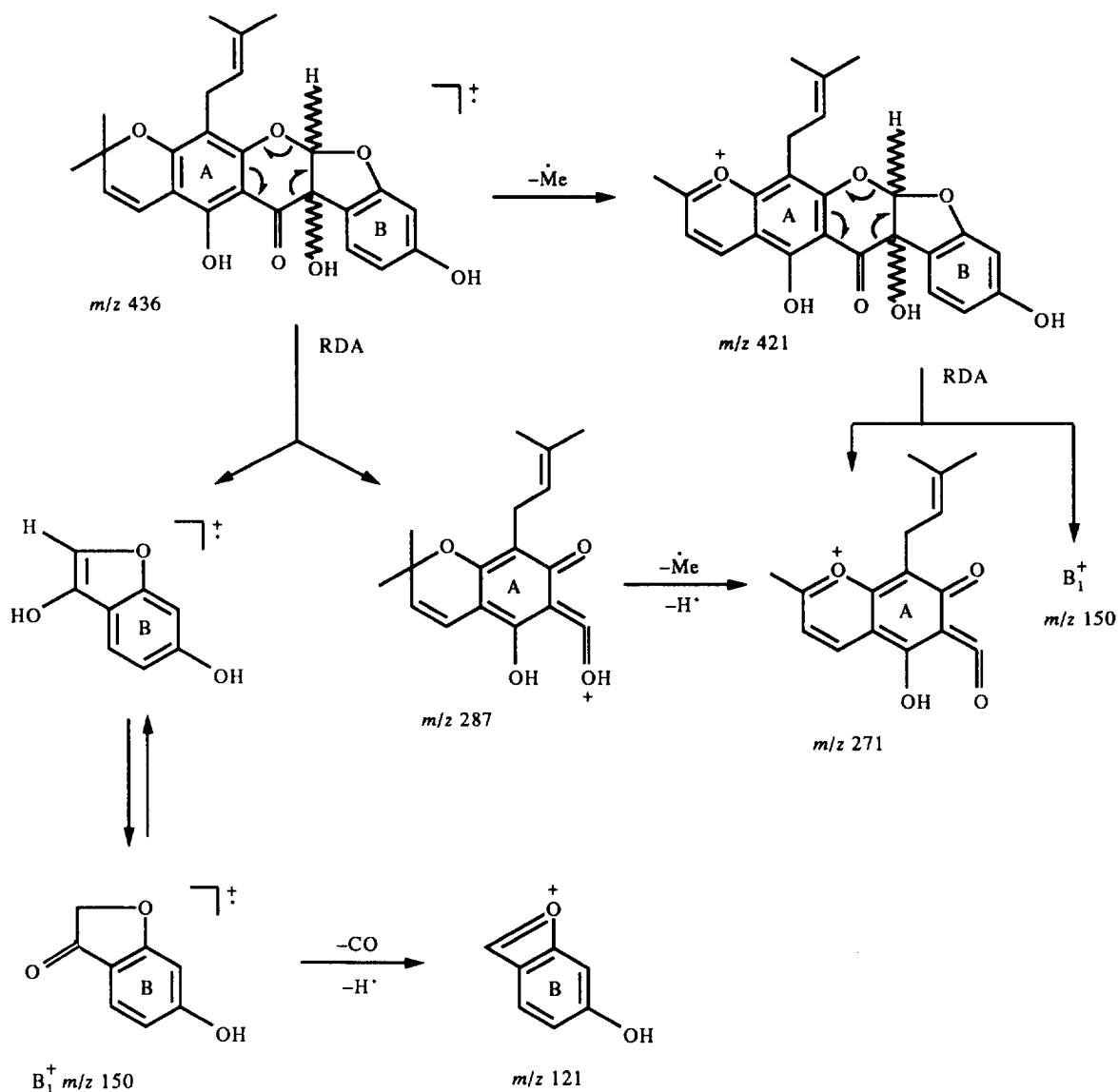
Scheme 1. Mass spectral fragmentation of 1.

In order to decide on the orientations of the two units on ring A, and to confirm the assignment of H-2, ^{13}C–^{1}H (HETCOR and COLOC) NMR techniques [11] were used. The COLOC spectrum showed correlations between H-2 (δ6.28) and C-4 (192.0), H-2 (6.28) and C-9 (161.1), H-1''' (3.22) and C-9 (161.1), H-1''' (3.22) and C-7 (156.5), H-4'' (6.58) and C-5 (155.8). The presence of asymmetric carbons (C-2 and C-3) in 1 justified its optical rotation although its stereochemistry was not determined. From the above spectroscopic studies, 1 was established as 3,5,4'-trihydroxy-8-(γ,γ-dimethylallyl)-2'',2''-dimethylpyrano [5'', 6'':6, 7] coumaronochromone.

Compound 2, erysenegalensein K, was obtained as a gum and its molecular formula $C_{22}H_{18}O_6$ was deduced from the CI- (*m/z* 379 [M + H]$^+$) and EI-mass spectra which showed [M]$^+$ at *m/z* 378. The compound gave a positive Mg–HCl (reddish) test suggesting the presence of

an isoflavonoid or flavonoid skeleton. The IR spectrum showed bands at 3400 (OH) and 1640 cm^{-1} (C = O chelated). In the ^{1}H NMR spectrum, the singlet observed at δ8.15 was characteristic of the isoflavone skeleton and was supported by its UV (MeOH) spectrum which showed an absorption maximum at 268 nm. The bathochromic shifts ($\Delta\lambda$ = 8 and 12 nm) observed in the UV spectrum by the addition of AlCl$_3$ and AlCl$_3$/HCl, respectively, suggested that 2 had a free hydroxyl group at the C-5 position. This was then confirmed by the singlet in the ^{1}H NMR spectrum at δ12.70, which exchanged on deuteration. The presence of a γ,γ-dimethylallyl group was indicated by a doublet at δ3.64 (2H, *d*, *J* = 7.0 Hz, H-1'''), a triplet at δ5.36 (1H, *t*, *J* = 7.0 Hz, H-2''') and two singlets at δ1.72 (Me-*trans*) and 1.88 (Me-*cis*) (Table 1); these assignments were confirmed by 2D ^{13}C–^{1}H NMR. There were also two doublets at δ7.64 (1H, *d*, *J* = 2.0 Hz,

H-2″) and 7.0 (1H, d, J = 2.0 Hz, H-3″) assigned to the two protons of the furano group fused on an aromatic ring, in agreement with the literature [12] and confirmed by the ^{13}C NMR spectrum which showed C-2″ and C-3″ signals, respectively, at δ144.4 and 103.7 (Table 1). The EI-mass spectrum showed important ion fragments at m/z 335 [M − C$_3$H$_7$]$^+$, 323 [M − C$_4$H$_7$]$^+$, 189 and 134. Those at m/z 189 and 134 resulted from retro-Diels–Alder of the more stable ion at m/z 323 and were more characteristic. The first one at m/z 189 confirmed that the two units (prenyl and furano) were linked to ring A at the C-6 and C-8 positions, since there was no signal at δ5.90–6.10 characteristic of protons at these positions. The second one at m/z 134 resulted from ring B which carried two hydroxyl groups at *meta*-positions (C-2′ and C-4′), as confirmed by the ^{1}H and ^{13}C NMR spectra; the three ring B protons H-3′, H-5′ and H-6′ chemical shifts were observed as an ABX system, respectively, at δ6.60 (1H, d, J = 2.0 Hz), 6.50 (1H, dd, J = 2.0, 8.0 Hz) and 7.05 (1H, d, J = 8.0 Hz), while the chemical shifts of C-2′ (δ152.7) and C-4′ (δ154.8) were in agreement with the literature [10].

In order to establish the exact orientations of the two groups on ring A, ^{13}C–^{1}H (HETCOR and COLOC) NMR techniques [11] were also used as in 1. In the COLOC spectrum, correlations observed between H-2 (δ8.15) and C-9 (157.1), H-1‴ (3.64) and C-9 (157.1), H-1‴ (3.64) and C-7 (159.1) confirmed that the prenyl group was linked at the C-8 position, while correlations between H-3″ (7.0) and C-5 (159.2), H-3″ (7.0) and C-7 (159.1) confirmed that the fusion of the furano group was linear. Therefore, from the above spectroscopic studies 2 was deduced to be 5, 2′, 4′-trihydroxy-8-(γ,γ-dimethylallyl)-furano [4″, 5″:6, 7] isoflavone.

From the ^{1}H and ^{13}C NMR spectral studies, the five other co-occurring compounds (3–7) were found to be pentacyclic triterpenoids of the olean-12-ene series [13]. Melting points, [α]$_D$, CI- and EI-mass spectral determinations permitted their identification as the known compounds, β-amyrin (3) [14], maniladiol (4) [15], erythrodiol (5) [14], oleanolic acid (6) [14] and cornulacic acid (7) [16].

EXPERIMENTAL

General. Mps: uncorr. Silica gel 60 H (5–40 μm), 60 C (20–40 μm) and 60 (70–230 mesh) were used for CC under compressed air (300 mbar), while silica gel 60 F 254 or 1500/LS 254 were used for TLC and prep. TLC. All NMR expts were performed at 300 and 75 MHz, respectively, for ^{1}H and ^{13}C. Samples were dissolved in CDCl$_3$ and chemical shifts were referenced to int. TMS for ^{1}H NMR and to deuterated solvents for ^{13}C NMR. For the 2D NMR COLOC experiments, an optimized sequence has been developed for correlation via long-range coupling, notably $^3J_{C-H}$ [11].

Plant material. Erythrina senegalensis DC ‘stem bark was collected at Foumban, West Cameroon in April 1988. Herbarium specimens documenting the collection are deposited at the National Herbarium, Yaounde.

Extraction and isolation. Dried and ground stem bark (17 kg) was extracted with MeOH and evapd. The crude MeOH extract was then re-extracted with CH$_2$Cl$_2$ to give 580 g of CH$_2$Cl$_2$ extract. Repeated CC on silica gel (cyclohexane, CH$_2$Cl$_2$, EtOAc and MeOH) and TLC permitted the regrouping of the resulting frs into series A–G. Purification of series E (62 g) by CC and prep. TLC (CH$_2$Cl$_2$–MeOH, 19:1) gave 1 while studies of series D (21 g) by the same method yielded 2.

Erysenegalensein J (1). Gum. [α]$_D^{20}$ + 32.0° (CHCl$_3$; c 0.130). IR ν_{max}^{KBr} cm^{-1}: 3400, 3300, 1640. UV λ_{max}^{MeOH} nm (log ε): 276 (4.11), 322 (3.85); + AlCl$_3$ nm (log ε): 276 (4.13), 322 (3.94); + AlCl$_3$–HCl nm (log ε): 276 (4.14), 322 (3.94). HRMS (Found 436.1817. C$_{25}$H$_{24}$O$_7$ requires: 436.1812). CIMS (NH$_3$, probe) 90 eV, m/z (rel. int.): 437 [M + H]$^+$ (100). EIMS (probe) 70 eV, m/z (rel. int.): 436 [M]$^+$ (22), 421 (35), 375 (8), 324 (20), 287 (48), 271 (100), 269 (27), 243 (35), 231 (45), 215 (20), 189 (12), 150 (12), 121 (20), 91 (25), 77 (33). ^{1}H NMR (300 MHz, CHCl$_3$) and ^{13}C NMR (75 MHz, CDCl$_3$) in Table 1.

Erysenegalensein K (2). Gum. IR ν_{max}^{KBr} cm^{-1}: 3400, 3300, 1640. UV λ_{max}^{MeOH} nm (log ε): 259 (4.12), 268 (4.11); + AlCl$_3$ nm (log ε): 260 (4.10), 276 (4.06); + AlCl$_3$–HCl nm (log ε): 232 (4.07), 280 (4.11). (Found: C, 69.9; H, 4.7. C$_{22}$H$_{18}$O$_6$ requires: C, 69.9; H, 4.8%). CIMS (NH$_3$ probe) 90 eV, m/z (rel. int.): 379 [M + H]$^+$ (100). EIMS (probe) 70 eV, m/z (rel. int.): 378 [M]$^+$ (52), 353 (18), 335 (65), 323 (100), 305 (20), 294 (22), 189 (6), 134 (5), 105 (8), 77 (20), 43 (75). ^{1}H NMR (300 MHz, CDCl$_3$) and ^{13}C NMR (75 MHz, CDCl$_3$) in Table 1.

β-Amyrin (3). Crystals. Mp 202°. [α]$_D^{20}$ + 85.0° (CHCl$_3$; c 1.30). IR, ^{1}H, ^{13}C NMR and EIMS were in agreement with ref. [14] values.

Maniladiol (4). Crystals. Mp 214°. [α]$_D^{20}$ + 68.0° (CHCl$_3$; c 0.18). IR, ^{1}H, ^{13}C NMR and EIMS corresponded to ref. [15] values.

Erythrodiol (5). Crystals. Mp 236°. [α]$_D^{20}$ + 76.0° (CHCl$_3$; c 0.30). IR, ^{1}H, ^{13}C NMR and EIMS were in agreement with lit. [14] values.

Oleanolic acid (6). Crystals. Mp 305°. [α]$_D^{20}$ + 80.0° (MeOH; c 0.17). IR, ^{1}H, ^{13}C NMR and EIMS agreed with ref. [14] values.

Cornulacic acid (7). Crystals. Mp 288°. [α]$_D^{20}$ + 75.0° (MeOH; c 0.15). IR, ^{1}H, ^{13}C NMR and EIMS were in agreement with ref. [16] values.

REFERENCES

1. Subba Raju, K. V., Srimannarayana, G., Ternai, B., Stanley, R. and Markham, K. R. (1981) *Tetrahedron* 37, 957.
2. Tahara, S., Ingham, J. L. and Mizutani, J. (1985) *Agric. Biol. Chem.* 49, 1775.
3. Tahara, S., Orihara, S., Ingham, J. L. and Mizutani, J. (1989) *Phytochemistry* 28, 901.

4. Mizuno, M., Matsuura, N., Tanaka, T., Tinuma, M. and Feng-chi, H. (1991) *Phytochemistry* **30**, 3095.
5. Wandji, J., Nkengfack, A. E., Fomum, Z. T., Ubrillas, R., Killday, K. B. and Tempesta, M. S. (1990) *J. Nat. Prod.* **53**, 1425.
6. Wandji, J., Fomum, Z. T., Tillequin, F., Seguin, E. and Koch, M. (1994) *Phytochemistry* **35**, 245.
7. Wandji, J., Fomum, Z. T., Tillequin, F., Skaltsounis, A. L. and Koch, M. (1994) *Planta Med.* **60**, 178.
8. Wandji, J., Fomum, Z. T., Tillequin, F., Baudouin, G. and Koch, M. (1994) *Phytochemistry* **35**, 1573.
9. Markham, K. R. and Mabry, T. J. (1975) in *The Flavonoids* (Harnorne, J. B., Mabry, T. J. and Mabry, H., eds), pp. 45–125. Chapman & Hall, London.
10. Agrawal, P. K. (ed.) (1989) *Carbon*-13 *NMR of Flavonoids.* Elsevier, Amsterdam.
11. Croasmum, W. R. and Carbon, M. K. (1987) in *Two Dimensional NMR Spectroscopy: Applications for Chemists and Biochemists,* pp. 199–201. VCH, New York.
12. Steck, W. and Mazurzk, M. (1972) *Lloydia* **35**, 418.
13. Doddrell, D. M., Pegg, B. T. and Bendall, M. R. (1982) *J. Magn. Reson.* **48**, 323.
14. Wehrli, F. W. and Nishida, T. (1979) *Fortschr. Chem. Org. Naturst.* **36**, 89.
15. Nkengfack, A, E., Fomum, Z. T., Ubrillas, R. and Tempesta, M. S. (1990) *J. Nat. Prod.* **52** 1252.
16. Dawidar, A. M., Reisch, J. and Amer, M. A. (1980) *Egypt. J. Chem.* **23**, 31.
17. Nkengfack, A. E., Vouffo, T. W., Fomum. Z. T., Meyer, M., Bergendorff, O and Sterner, O. (1994) *Phytochemistry* **36**, 1047.