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Flavones and isoflavones from the west African Fabaceae *Erythrina vogelii*

Alain F. Kamdem Waffo [a,b,c], Philip H. Coombes [a], Dulcie A. Mulholland [a,d,*]
Augustin E. Nkengfack [c], Zacharias T. Fomum [c]

[a] *Natural Products Research Group, School of Chemistry, University of KwaZulu-Natal, Howard College Campus, 4041, Durban, South Africa*
[b] *Department of Chemistry, Faculty of Science, University of Douala, P.O. Box 24157 Douala, Cameroon*
[c] *Department of Organic Chemistry, Faculty of Science, University of Yaounde I, P.O. Box 812 Yaounde, Cameroon*
[d] *School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, UK*

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Abstract

The CH_2Cl_2/MeOH extract of the stem bark of *Erythrina vogelii* (Fabaceae) from Nigeria has yielded two novel isoflavones, 7,4′-dihydroxy-8-(γ,γ-dimethylallyl)-2″ξ-(4″-hydroxy*iso*propyl)dihydrofurano[1″,3″:5,6]isoflavone (vogelin H) (**1**) and 7,4′-dihydroxy-8-[(2‴ξ, 3‴-dihydroxy-3‴-methyl)butyl]-2″,2″-dimethyl-3″,4″-dehydropyrano[1″,4″:5,6]isoflavone (vogelin I) (**2**), a novel flavone, 7,4′-dihydroxy-2″,2″-dimethyl-3″,4″-dehydropyrano[1″,4″:5,6]flavone (vogelin J) (**3**), and eight known flavonoids.

Keywords: Erythrina vogelii; Fabaceae; Stem bark; Isolation; Isoflavones; Flavones; 7,4′-Dihydroxy-8-(γ,γ-dimethylallyl)-2″ξ-(4″-hydroxy*iso*propyl)-dihydrofurano[1″,3″:5,6]isoflavone (Vogelin H); 7,4′-Dihydroxy-8-[(2‴ξ,3‴-dihydroxy-3‴-methyl)butyl]-2″,2″-dimethyl-3″,4″-dehydropyrano[1″,4″:5,6]-isoflavone (Vogelin I); 7,4′-Dihydroxy-2″,2″-dimethyl-3″,4″-dehydropyrano[1″,4″:5,6]flavone (Vogelin J); 6,8-Diprenylgenistein; 8-Prenylluteone; Warangalone; Scandenone; Auriculatin; 2,3-Dihydroauriculatin; Carpachromene

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1. Introduction

The genus *Erythrina* L. (Fabaceae), comprising approximately 130 species of “coral trees” distributed throughout the tropical and subtropical regions of the world, has been widely studied. More than 340 extractives have been isolated to date, with some 30 of these secondary metabolites variously reported to display antimicrobial (Ingham and Markham, 1980; Kamat et al., 1981; Mitscher et al., 1988a,b; Chacha et al., 2005), antibacterial (Fomum et al., 1986), antifungal (Tahara et al., 1984; Bojase et al., 2001), anti-inflammatory (Wandji et al., 1994; Chacha et al., 2005), antiemetic and antitussive (Abbasoglu et al., 1991), and cytotoxic (Hou et al., 2001) properties, and also to act as phytoalexins (Dagne et al., 1993) and phospholipase A_2 inhibitors (Hegde et al., 1997).

Previous studies (Atindehou et al., 2002; Queiroz et al., 2002) on the root bark of *Erythrina vogelii* Hook. from Ivory Coast have yielded vogelins A–G, seven new ring B prenylated isoflavonoids, and five known isoflavonoids.

2. Results and discussion

In continuation (Fomum and Ayafor, 1983; Kamdem Waffo, 2000; Njamen et al., 2004) of our studies on species of this genus, we now report on the isolation of two novel isoflavones and a novel flavone, together with eight known flavonoids, from the CH_2Cl_2/MeOH (1:1) extract of the stem bark of *E. vogelii* collected in Nigeria.

Compound **1** was assigned a molecular formula of $C_{25}H_{26}O_6$ on the basis of HREIMS data, and showed a

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[*] Corresponding author. Tel.: +27 31 260 1395; fax: +27 31 260 3091.
E-mail addresses: mulholld@ukzn.zc.za, d.mulholland@surrey.ac.uk (D.A. Mulholland).

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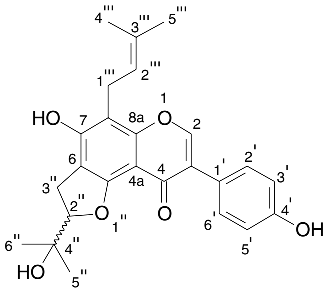
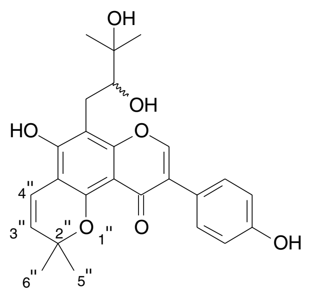
λ_{max} at 271 nm in the UV spectrum. A 1H singlet resonance at δ_H 7.75 and corresponding olefinic oxymethine signal at δ_C 152.5 are characteristic of H-2 and C-2, respectively, in an isoflavone skeleton (Mabry et al., 1970; Agrawal, 1989). From a pair of coupled doublets at δ_H 7.28 and 6.83 (each 2H, $J = 8.5$ Hz, H-2′/H-6′, H-3′/H-5′); δ_C 130.2 (each CH, C-2′/6′), 115.4 (each CH, C-3′/5′) was deduced the presence of a *para* disubstituted ring B, while that of a hydroxyl group at C-4′ was established from a peak at *m/z* 118 in the EIMS. Further inspection of the NMR and mass spectra showed compound **1** to possess a γ,γ-dimethylallyl (prenyl) substituent (*m/z* 379 $[M - 43]^+$ and 367 $[M - 55]^+$; δ_H 3.29 (2H, *d*, $J = 7.3$ Hz, 2H-1‴), 5.23 (1H, *m*, H-2‴), 1.64 (3H, *s*, 3H-4‴), 1.73 (3H, *s*, 3H-5‴); δ_C 21.8 (CH_2, C-1‴), 121.4 (CH, C-2‴), 132.2 (C, C-3‴), 17.7 (CH_3, C-4‴), 25.5 (CH_3, C-5‴)). The presence of a hydroxy*iso*propyl-dihydrofuran ring, suggested by characteristic peaks in the EIMS at *m/z* 363 $[M - 59]^+$ and 59 (Tahara et al., 1984, 1989), was supported by the observation of resonances attributable to two non-equivalent geminal methyl groups (δ_H 1.20/1.30 (each 3H, *s*, 3H-5″/6″); δ_C 25.5/24.0 (each CH_3, C-5″/6″)), an oxymethine proton (δ_H 4.74 (1H, *d*, $J = 8.3$ Hz, H-2″); (δ_C 91.0 (CH, C-2″)), two diastereotopic protons (δ_H 3.20 (2H, *br d*, $J = 8.1$ Hz, 2H-3″; δ_C 27.1 (CH_2, C-3″)) and a fully substituted oxygenated carbon (δ_C 72.2 (C, C-4″)) (Tahara et al., 1989).

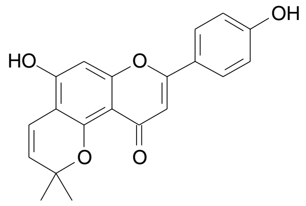
The absence of signals in the region δ_H 5.7 – 6.1, normally attributed to H-6 and H-8, placed both the prenyl group and dihydrofuran ring on ring A, while the lack of both the downfield singlet at δ_H 12.0–13.2 and the absorption band at 3280 cm^{-1} in the IR spectrum, characteristic of a chelated OH group at C-5, suggested the latter substituent to be fused at C-5/C-6, and thus, given the normal biosynthetic requirement of a hydroxy group at C-7, that the prenyl group be located at C-8. This placement was supported by correlations in the HMBC spectrum (Fig. 1) between both the H-2 and 2H-1‴ resonances and a downfield fully substituted carbon signal at δ_C 151.8, which can only be C-8a, and by further correlations between both 2H-1‴and 2H-3″ to a second downfield fully substituted carbon signal at δ_C 164.5, which must then be C-7. Correlations between the 2H-3″ resonance and fully substituted carbon signals at δ_C 101.8 and 159.7, assigned to C-6 and C-5, respectively, and between H-2″ and both of these, confirmed these assignments. Compound **1** is thus the novel 7,4′-dihydroxy-8-(γ,γ-dimethylallyl)-2″ξ-(4″-hydroxy*iso*propyl)dihydrofurano[1″,3″:5,6]isoflavone, which we name vogelin H, and is regioisomeric with senegalensin, from *E. senegalensis* DC. (Wandji et al., 1990), euchrenone b_{10}, from *Euchresta horsfieldii* (Lesch.) Bennet (Mizuno et al., 1990), and lupinisoflavone G, from *Lupinis albus* L. (Tahara et al., 1989) and *Derris scandens* Benth. (Sekine et al., 1999); the structures of the former two compounds, transposed when originally reported, were revised on recent isolation from *E. suberosa* var *glabresence* Haines (Tanaka et al., 2001).



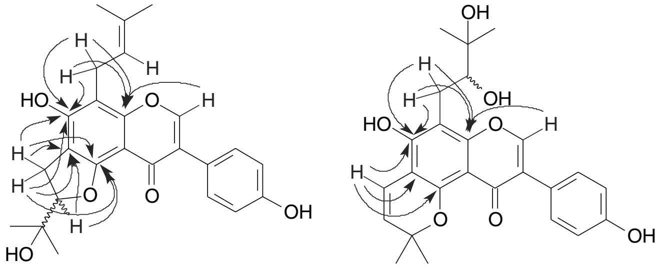
vogelin H (**1**)



vogelin I (**2**)



vogelin J (**3**)



(**1**) (**2**)

Fig. 1. Selected HMBC correlations in vogelins H (**1**) and I (**2**).

In similar fashion, compound **2**, assigned the molecular formula $C_{25}H_{26}O_7$ by HREIMS, possesses an isoflavone skeleton (δ_H 8.01 (1H, *s*, H-2); δ_C 154.2 (CH, C-2)) and a *para* disubstituted C-4′-hydroxy ring B (δ_H 7.32 and 6.84 (each 2H, $J = 8.6$ Hz, H-2′/6′, H-3′/5′); δ_C 130.9 (each CH, C-2′/6′), 116.0 (each CH, C-3′/5′)). However, the signals of both the prenyl group and dihydrofuran ring in vogelin H (**1**) were absent, having been replaced by those attributable to a 2″,2″-dimethyl-3″,4″-dehydropyran ring (δ_H 5.64 (1H, *d*, $J = 10.0$ Hz, H-3″), 6.67 (1H, *d*, $J = 10.0$ Hz, H-4″), 1.46 (6H, *s*, 3H-5″/3H-6″); δ_C 78.9 (C, C-2″), 128.7 (CH, C-3″), 116.1 (CH, C-4″), 28.5/28.5 (each CH_3, C-5″/C-6″)), and a 2ξ, 3-dihydroxy-3-methylbutyl group (δ_H 3.60 (1H, *m*, H-2‴), 2.88 (1H, *m*, H-1a‴), 2.85 (1H, *m*, H-1b ‴), 1.28 (6H, *s*, 3H-4‴/3H-5‴); δ_C 25.5 (CH_2, C-1‴), 78.6 (CH, C-2‴), 73.6 (C, C-3 ‴), 24.4/26.0 (each CH_3, C-4‴/C-5‴)) (Takashima and Ohsaki, 2002). As in vogelin H (**1**), the absence of the resonances normally attributable to H-6 and H-8 placed both of these groups on the A ring. Correlations in the HMBC spectrum (Fig. 1) between both H-2 and 2H-1‴and a downfield fully substituted carbon resonance at δ_C 156.4, which must be C-8a, and between 2H-1‴and a second downfield fully substituted carbon resonance at δ_C 158.1, which must then be C-7, place the 2ξ,3-dihydroxy-3-dimethylbutyl group at C-8.

Further correlations between H-4″ and C-7, and between H-4″ and fully substituted carbon signals at δ_C 105.5 and 155.5, assigned to C-6 and C-5, respectively, locate the dehydropyran ring at C-5/C-6. Compound **2** is thus the novel 7,4′-dihydroxy-8-[(2‴ξ,3‴-dihydroxy-3‴-methyl)butyl]-2″,2″-dimethyl-3″,4″-dehydro-pyrano[1″,4″:-5,6]isoflavone, which we name vogelin I.

The UV and NMR spectra of vogelin J (**3**), assigned the molecular formula $C_{20}H_{16}O_5$ from HREIMS data, displayed λ_{max} peaks at 272, 313 and 330 nm, a 1H singlet resonance at δ_H 6.46, and corresponding upfield olefinic signal at δ_C 103.1 (CH) characteristic of H-3 and C-3, respectively, in a flavone skeleton (Mabry et al., 1970; Agrawal, 1989). In common with vogelins H (**1**) and I (**2**), vogelin J (**3**) possesses a *para* disubstituted 4′-hydroxy ring B (δ_H 7.60 and 6.85 (each 2H, $J = 8.9$ Hz, H-2′/H-6′, H-3′/H-5′); δ_C 127.7 (each CH, C-2′/6′), 116.2 (each CH, C-3′/5′)) and a 2″,2″-dimethyl-3″,4″-dehydropyran ring (δ_H 5.54 (1H, *d*, $J = 10.0$ Hz, H-3″), 6.62 (1H, *d*, $J = 10.0$ Hz, H-4″), 1.40 (6H, *s*, 3H-5″/3H-6″); δ_C 78.0 (C, C-2″), 127.3 (CH, C-3″), 115.7 (CH, C-4″), 27.9/28.0 (each CH_3, C-5″/C-6″)). The latter was placed at C-5/C-6 when a bathochromic shift with NaOAc, but not $AlCl_3$, indicated the presence of a hydroxyl group at C-7 only. Vogelin J (**3**) is thus 7,4′-dihydroxy-2″,2″-dimethyl-3″,4″-dehydropyrano[1″,4″:5,6]flavone, a novel regioisomer of limonianin from *Citrus limon* [L.] Burn. (Chang, 1990), carpachromene from *Flindersia laevicarpa* C.T.White (Picker et al., 1976; Jain et al., 1978) and yinyanghuo C from *Vancouveria hexandra* (Hook.) C. Morren & Decne (Linuma et al., 1993) and *Epimedium sagittatum* (Siebold & Zucc.) Maxim. (Chen et al., 1996).

The known compounds were identified as 6-prenylapigenin (Abegaz et al., 1998), 6,8-diprenylgenistein (Shirataki et al., 1982), 8-prenylluteone (Nkengfack et al., 1989), warangalone (scandenone) (Nkengfack et al., 1989), auriculatin (Shabbir et al., 1968; Subba Raju et al., 1981), 2,3-dihydroauriculatin (Shabbir et al., 1968; Taylor et al., 1986), limonianin (Chang, 1990) and carpachromene (Saraswathy et al., 1998) by comparison of their physical properties and spectral data with the literature values.

3. Experimental

3.1. General

Melting points were determined on a Kofler micro-hot stage melting point apparatus and are uncorrected. NMR spectra were recorded at room temperature on a 400 MHz Varian UNITY-INOVA spectrometer. Chemical shifts are expressed in δ (ppm) units relative to tetramethylsilane (TMS) as internal standard and coupling constants are given in Hz. ^1H NMR, ^{13}C, HMBC, HSQC and NOESY spectra were recorded in $CDCl_3$ and CD_3OD. UV spectra were obtained on a Varian DMS 300 UV–visible spectrometer with MeOH as solvent. IR spectra were recorded on a Nicolet Impact 400D Fourier-Transform Infrared (FT-IR) spectrometer, using NaCl windows with $CHCl_3$ as solvent against an air background. LREIMS and HREIMS were taken on Perkin–Elmer 6890-Agilent 5975 GC–MS and Micromass VG 70 SEQ instruments, respectively. Optical rotations were measured at room temperature in $CHCl_3$ on a Perkin–Elmer 341 Polarimeter, using a 100 mm quartz microcell flow tube.

3.2. Plant material

Erythrina vogelii Hook. was collected at Ogbomoso, Nigeria, in May 2003 and identified at the University of Ibadan Herbarium and the Cameroon National Herbarium, Yaounde, where a voucher specimen (20693/SRF Cam.) is retained for verification purposes.

3.3. Extraction and isolation of compounds

The air-dried, ground stem bark material of *E. vogelii* (3 kg) was extracted for 72 h at room temperature with CH_2Cl_2:MeOH (1:1) and concentrated under reduced pressure to give 201.3 g of extract. Repeated combinations of vacuum liquid and gravity column chromatography on Merck 7729 and 9385 silica gels, and PTLC on aluminium backed analytical TLC (Merck 5554) plates, using various mixtures of hexane:EtOAc:MeOH, afforded vogelins H (**1**) (4.0 mg), I (**2**) (4.9 mg) and J (**3**) (15.2 mg), together with 6-prenylapigenin (6.8 mg), 6,8-diprenylgenistein (9.7 mg), 8-prenylluteone (10.2 mg), warangalone (scandenone) (9.0 mg), auriculatin (10.0 mg), 2,3-dihydroauriculatin (10.1 mg), limonianin (6.7 mg) and carpachromene (6.8 mg).

3.3.1. 7,4′-Dihydroxy-8-(γ,γ-dimethylallyl)-2″ξ-(4″-hydroxyisopropyl)dihydrofurano-[1″,3″:5,6]isoflavone, vogelin H (1)

Pale yellow powder; m.p. 195–197 °C; $[\alpha]_D^{20} = -38°$ (*c*, 0.0015 in $CHCl_3$); ν_{max}(NaCl) cm^{-1} 3545, 1642, 1610, 1512, 1425, 1382, 1270, 1215, 1172, 1075, 836; HREIMS (70 eV) *m/z* 422.1720 (calc. for $C_{25}H_{26}O_6$ 422.1729); EIMS (70 eV) *m/z* (rel. int.) 422 (98), 407 (20), 379 (70), 367(100), 363 (25), 352 (33), 349 (28), 335 (18), 320 (14), 307 (30), 295 (40), 118 (35), 59 (10); λ_{max} (MeOH) nm (log ε): 203 (4.48), 216 (4.37), 271 (4.48), (MeOH + NaOAc) 275; ^1H NMR spectral data (400 MHz, CD_3OD) δ_H 7.75 (1H, *s*, H-2), 7.28 (2H, *d*, $J = 8.5$ Hz, H-2′/H-6′), 6.83 (2H, *d*, $J = 8.5$ Hz, H-3′/H-5′), 5.23 (1H, *m*, H-2‴), 4.74 (1H, *d*, $J = 8.3$ Hz, H-2″), 3.29 (2H, *d*, $J = 7.3$ Hz, 2H-1‴), 3.20 (2H, *br d*, $J = 8.1$ Hz, 2H-3″), 1.73 (3H, *s*, 3H-5‴), 1.64 (3H, *s*, 3H-4‴), 1.30/1.20 (each 3H, *s*, 3H-5″/6″); ^{13}C NMR spectral data (100 MHz, CD_3OD) (Table 1).

3.3.2. 7,4′-Dihydroxy-8-[(2‴ξ,3‴-dihydroxy-3‴-methyl)butyl]-2″,2″-dimethyl-3″,4″-de-hydropyrano[1″,4″:5,6]isoflavone, vogelin I (2)

Pale yellow powder; m.p. 248–249 °C; $[\alpha]_D^{20} = 42°$ (*c*, 0.0050 in $CHCl_3$); ν_{max}(NaCl) cm^{-1} 3525, 1640, 1610,

Table 1
^{13}C NMR spectral data for vogelins H (**1**), I (**2**), and J (**3**) (100 MHz)

Carbon	1	2	3
2	152.5 (CH)	154.2 (CH)	164.5 (C)
3	123.0 (C)	124.0 (C)	103.1 (CH)
4	180.7 (C)	182.4 (C)	182.5 (C)
4a	104.0 (C)	105.8 (C)	102.8 (C)
5	159.7 (C)	155.5 (C)	155.7 (C)
6	101.8 (C)	105.5 (C)	105.4 (C)
7	164.5 (C)	158.1 (C)	159.3 (C)
8	107.0 (C)	106.5 (C)	95.2 (CH)
8a	151.8 (C)	156.4 (C)	156.9 (C)
1′	121.4 (C)	122.7(C)	122.0(C)
2′	130.2(CH)	130.9(CH)	127.7 (CH)
3′	115.4 (CH)	116.0 (CH)	116.2 (CH)
4′	156.0 (C)	158.0 (C)	160.8 (C)
5′	115.4 (CH)	116.0 (CH)	116.2 (CH)
6′	130.2(CH)	130.9(CH)	127.7 (CH)
2″	91.0 (CH)	78.9 (C)	78.0 (C)
3″	27.1 (CH$_2$)	128.7 (CH)	127.3 (CH)
4″	72.2 (C)	116.1 (CH)	115.7 (CH)
5″	25.5 (CH$_3$)^{A}	28.5 (CH$_3$)	28.0 (CH$_3$)^{A}
6″	24.0 (CH$_3$)^{A}	28.5 (CH$_3$)	27.9 (CH$_3$)^{A}
1‴	21.8 (CH$_2$)	25.5 (CH$_2$)	
2‴	121.4 (CH)	78.6 (CH)	
3‴	132.2 (C)	73.6 (C)	
4‴	17.7 (CH$_3$)	26.0 (CH$_3$)^{A}	
5‴	25.5 (CH$_3$)	24.4 (CH$_3$)^{A}	

^{A} Values interchangeable within column.

1085 and 840; HREIMS (70 eV) *m*/*z* 438.1665 (calc. for $C_{25}H_{26}O_7$ 438.1678); EIMS (70 eV) *m*/*z* (rel. int.) 438 (46), 423 (64), 398 (3), 379 (29), 349 (100), 335 (20), 321 (60), 295 (13), 236 (5), 203 (8), 166 (9), 152 (7), 118 (4), 91 (18), 57 (27), 28 (61); λ_{max} (MeOH) nm (log ε): 215 (4.41), 268 (4.40), 294 (3.93); ^{1}H NMR spectral data (400 MHz, CDCl$_3$) δ_H 8.01 (1H, *s*, H-2), 7.32 (2H, *d*, *J* = 8.6 Hz, H-2′/H-6′), 6.84 (2H, *d*, *J* = 8.5 Hz, H-3′/H-5′), 6.67 (1H, *d*, *J* = 10.0 Hz, H-4″), 5.64 (1H, *d*, *J* = 10.0 Hz, H-3″), 3.60 (1H, *m*, H-2‴), 2.88 (1H, *m*, H-1a‴), 2.85 (1H, *m*, H-1b‴), 1.46 (6H, *s*, 3H-5″/3H-6″), 1.28 (6H, *s*, 3H-4‴/3H-5‴), ^{13}C NMR spectral data (100 MHz, CDCl$_3$) (Table 1).

3.3.3. 4′,7-Dihydroxy-2″,2″-dimethyl-3″,4″-dehydropyrano[1″,4″:5,6]flavone, vogelin J (3)

Pale yellow needles; m.p. 238–239 °C; v_{max}(NaCl) cm^{-1} 3450, 1652, 1589, 1540, 1400, 1272, 1235; HREIMS (70 eV) *m*/*z* 336.0932 (calc. for $C_{20}H_{16}O_5$ 336.0998); EIMS (70 eV) *m*/*z* (rel. int.) 336 (25), 321 (100), 203 (19), 135 (27), 118 (43); λ_{max} (MeOH) nm (log ε): 236 (4.42), 272 (4.36), 313 (4.23), 330 (4.25), 356 (3.78), (MeOH + NaOAc) 277; ^{1}H NMR spectral data (400 MHz, CDCl$_3$)δ_H 7.60 (2H, *d*, *J* = 8.9 Hz, H-2′/6′), 6.85 (2H, *d*, *J* = 8.9 Hz, H-3′/H-5′), 6.62 (1H, *d*, *J* = 10.0 Hz, H-4″), 6.46 (1H, *s*, H-3), 6.34 (1H, *s*, H-8), 5.54 (1H, *d*, *J* = 10.0 Hz, H-3″), 1.40 (6H, *s*, 3H-5″/3H-6″); ^{13}C NMR spectral data (100 MHz, CDCl$_3$) (Table 1).

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