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# Flavone glycosides from *Uraria crinita*

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***Abstract:***

**Four flavone glycosides including apigenin 7-*O*-*β*-glucoside (1), chrysoeriol 7-*O*-*β*-glucoside (2), rhoifolin (3), and 3′-methoxyapiin (4) were isolated from the n-butanol extract of the whole plant *Uraria crinita* collected in the Phu Tho province, Vietnam. Their structures were elucidated by 1D- and 2D-NMR spectra and compared with those reported in the literature. Compounds 1-3 were obtained from the genus *Uraria* for the first time.**

***Keywords:*** **apigenin 7-*O*-*β*-glucoside, chrysoeriol 7-*O*-*β*-glucoside, flavone glycosides, rhoifolin, *Uraria crinita*.**

***Classification number:*** **2.2**

## Introduction

*Uraria crinita* (L.) DC. (Leguminosae) is an edible herb that is widely distributed across all regions of Vietnam. It has been consumed as a vegetable or used in folk medicine for the treatment of rheumatism, diarrhoea, sprains, injuries, and lung diseases [1]. Pharmacological investigations have demonstrated that *U. crinita* has various therapeutic properties including antioxidant, anti-inflammatory, and osteogenic activities as well as reduce stress-induced ulcers [2, 3]. Several compounds including flavonoids, triterpenes, megastigmanes, and nucleosides have been reported to be active components in this plant [3-6]. Our previous studies on *U. crinita* has led us to isolate four new phenolics, (3*S*)-5,7-dihydroxy-2′,3′,4′-trimethoxy-6,5′-diprenylisoflavanone, 3,5,7,2’,4’-pentahydroxyisoflavanone, 3,4-dimethoxyphenyl 1-*O*-(6′-*O*-acetyl)-*β*-D-glucopyranoside, and 3,4,5-trimethoxyphenyl 1-*O*-(6′-*O*-acetyl)-*β*-D-glucopyranoside, along with eleven known compounds [7, 8]. Here, we report the isolation and structural determination of four flavone glycosides **1-4** from the n-butanol extract of the whole plant *U. crinita*.

## Materials and methods

### *General experimental procedures*

Nuclear magnetic resonance (NMR) spectra were taken on a Bruker Avance III 500 spectrometer (Bruker, Fällanden, Switzerland) and electrospray ionisation mass spectrometry (ESI-MS) was conducted on an Agilent LC-MSD-Trap-SL spectrometer (Varian, USA). Infrared (IR) spectra were performed on Perkin Elmer Spectrum Two IR spectrometer (Perkin Elmer, Waltham, MA, USA). Column chromatography was carried out on silica gel 60 (0.040-0.063 mm, Merck, Darmstadt, Germany), Sephadex LH-20 (Amersham Pharmacia Biotech, Tokyo, Japan), and Rp-18 (30-50 µm, Fuji Silysia Chemical Ltd, Aichi, Japan). Thin layer chromatography was performed on silica gel 60F254 (0.25 mm, Merck, Darmstadt, Germany). The spots on the plates were observed under UV light and by spraying with a solution of vanillin/sulfuric acid and 5 min of heating.

### *Plant material*

The whole plant *U. crinita* was collected in the Phu Tho province, Vietnam, in May 2019. The botanical identification was made by D. H. Thu, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). A voucher specimen (UC05/2019) was deposited at the Laboratory of Natural Products Research, Institute of Chemistry, VAST, Hanoi, Vietnam.

### *Extraction and isolation*

The dried whole plant *U. crinita* (12 kg) was ground and extraction was performed three times with methanol-water (95:5, v/v) at room temperature. The extracted solution was concentrated under reduced pressure in a rotary evaporator at 45°C to remove the methanol. The obtained crude extract was suspended in water and successively partitioned with

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n-hexane, ethyl acetate, and n-butanol, respectively. The organic solvents were evaporated to yield extracts of 138.6, 81.2, and 117.3 g, respectively.

The n-butanol extract (115 g) was subjected to silica gel chromatography eluted with a $CH_2Cl_2$-MeOH-$H_2O$ system (from 10:0:0 to 7:3:1, v/v) to give 10 fractions. Fraction 3 was repeatedly chromatographed on a silica gel column ($CH_2Cl_2$-MeOH-$H_2O$, 4:1:0.1, v/v) and then on a Sephadex LH-20 column (MeOH) to give compounds **1** (6 mg) and **2** (4 mg). Fraction 6 was purified on a silica gel column ($CH_2Cl_2$-MeOH-$H_2O$, 3:1:0.1, v/v) and then on an Rp-18 column (MeOH-$H_2O$, 2.5:2, v/v) to yield compound **3** (7 mg). Fraction 7 was measured using silica gel chromatography, eluted with $CH_2Cl_2$-MeOH-$H_2O$ (3:1:0.1, v/v), and then on Sephadex LH-20 column (MeOH) to give compound **4** (4 mg).

**Apigenin 7-*O*-*β*-glucoside (1):**

Yellow solid. $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta_H$ 12.96 (1H, s, 5-OH), 7.96 (2H, d, *J*=9.0 Hz, H-2′, H-6′), 6.94 (2H, d, *J*=9.0 Hz, H-3′, H-5′), 6.86 (1H, s, H-3), 6.83 (1H, d, *J*=1.5 Hz, H-8), 6.45 (1H, d, *J*=1.5 Hz, H-6), 5.39 (1H, br s, OH), 5.12 (1H, br s, OH), 5.06 (1H, d, *J*=7.0 Hz, H-1″), 5.06 (1H, br s, OH), 4.61 (1H, m, OH), 3.72 (1H, d, *J*=10.5 Hz, H-6a″), 3.49-3.17 (5H, m, H-2″-H-6b″). $^{13}$C-NMR (125 MHz, $CD_3OD$): $\delta_C$ 182.03 (C-4), 164.25 (C-2), 162.96 (C-7), 161.35 (C-4′), 161.08 (C-5), 156.90 (C-9), 128.59 (C-2′, C-6′), 120.99 (C-1′), 115.98 (C-3′, C-5′), 105.33 (C-10), 103.11 (C-3), 99.88 (C-1″), 99.49 (C-6), 94.88 (C-8), 77.15 (C-5″), 76.44 (C-3″), 73.10 (C-2″), 69.58 (C-4″), 60.60 (C-6″). ESI-MS *m/z* 433.3 $[M + H]^+$, $C_{21}H_{20}O_{10}$. IR (KBr) $\nu_{max}$: 3390 (O-H), 2923 (C-H), 1635 (C=O), 1600, 1510, 1462 (C=C), 1205, 1143 (C-O) cm$^{-1}$.

**Chrysoeriol 7-*O*-*β*-glucoside (2):**

Yellow solid. $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta_H$ 12.96 (1H, s, 5-OH), 7.60 (1H, dd, *J*=8.5, 1.5 Hz, H-6′), 7.59 (1H, d, *J*=1.5 Hz, H-2′), 6.98 (1H, s, H-3), 6.95 (1H, d, *J*=8.5 Hz, H-5′), 6.86 (1H, d, *J*=2.0 Hz, H-8), 6.45 (1H, d, *J*=2.0 Hz, H-6), 5.39 (1H, br s, OH), 5.12 (1H, br s, OH), 5.06 (1H, d, *J*=7.0 Hz, H-1″), 5.06 (1H, br s, OH), 4.61 (1H, m, OH), 3.89 (3H, s, 3′-$OCH_3$), 3.72 (1H, d, *J*=10.5 Hz, H-6a″), 3.49-3.17 (5H, m, H-2″-H-6b″). $^{13}$C-NMR (125 MHz, $CD_3OD$): $\delta_C$ 182.03 (C-4), 164.14 (C-2), 162.96 (C-7), 161.08 (C-5), 156.90 (C-9), 150.93 (C-4′), 148.04 (C-3′), 121.31 (C-1′), 120.49 (C-6′), 115.76 (C-5′), 110.30 (C-2′), 105.33 (C-10), 103.43 (C-3), 99.99 (C-1″), 99.49 (C-6), 95.01 (C-8), 77.23 (C-5″), 76.44 (C-3″), 73.10 (C-2″), 69.58 (C-4″), 60.60 (C-6″), 55.96 (3′-$OCH_3$). ESI-MS *m/z* 463.4 $[M + H]^+$, $C_{22}H_{22}O_{11}$. IR (KBr) $\nu_{max}$: 3405 (O-H), 2906 (C-H), 1648 (C=O), 1612, 1506, 1453 (C=C), 1185, 1126, 1087 (C-O) cm$^{-1}$.

**Rhoifolin (3):**

White solid. $^1$H-NMR (500 MHz, $CD_3OD$): $\delta_H$ 7.90 (2H, d, *J*=9.0 Hz, H-2′, H-6′), 6.96 (2H, d, *J*=9.0 Hz, H-3′, H-5′), 6.81 (1H, d, *J* = 2.0 Hz, H-8), 6.68 (1H, s, H-3), 6.48 (1H, d, *J*=2.0 Hz, H-6), 5.31 (1H, d, *J*=2.0 Hz, H-1‴), 5.22 (1H, d, *J*=8.0 Hz, H-1″), 3.97-3.41 (C<u>H</u>-OH, C<u>H$_2$</u>-OH sugar), 1.35 (1H, d, *J*=6.0 Hz, H-6‴). $^{13}$C-NMR (125 MHz, $CD_3OD$): $\delta_C$ 184.06 (C-4), 166.82 (C-2), 164.43 (C-7), 162.96 (C-4′, C-5), 159.01 (C-9), 129.65 (C-2′, C-6′), 123.07 (C-1′), 117.08 (C-3′, C-5′), 107.14 (C-10), 104.15 (C-3), 102.54 (C-1‴), 101.08 (C-6), 99.94 (C-1″), 95.95 (C-8), 79.11 (C-2″), 79.02 (C-5″), 78.36 (C-3″), 73.96 (C-4‴), 72.22 (C-4″, C-3‴), 71.41 (C-2‴), 70.02 (C-5‴), 62.44 (C-6″), 18.23 (C-6‴). ESI-MS *m/z* 579.2 $[M + H]^+$, $C_{27}H_{30}O_{14}$. IR (KBr) $\nu_{max}$: 3458 (O-H), 2908 (C-H), 1653(C=O), 1600, 1452 (C=C), 1210, 1148 (C-O) cm$^{-1}$.

**3′-Methoxyapiin (4):**

Yellow solid. $^1$H-NMR (500 MHz, $CD_3OD$): $\delta_H$ 7.56 (1H, d, *J*=8.5 Hz, H-6′), 7.52 (1H, s, H-2′), 6.95 (1H, d, *J*=8.5, H-5′), 6.83 (1H, brs, H-8), 6.69 (1H, H-3), 6.48 (1H, d, *J*=1.5 Hz, H-6), 5.48 (1H, d, *J*=1.5 Hz, H-1‴), 5.17 (1H, d, *J*=7.5 Hz, H-1″), 4.07-3.41 (C<u>H</u>-OH, C<u>H$_2$</u>-OH sugar). $^{13}$C-NMR (125 MHz, $CD_3OD$): $\delta_C$ 184.05 (C-4), 166.67 (C-2), 164.66 (C-7), 162.91 (C-5), 158.98 (C-9), 152.36 (C-4′), 149.55 (C-3′), 123.51 (C-1′), 122.00 (C-6′), 116.83 (C-5′), 110.94 (C-1‴), 110.83 (C-2′), 107.11 (C-10), 104.50 (C-3), 101.07 (C-6), 100.29 (C-1″), 96.11 (C-8), 80.71 (C-3‴), 78.82 (C-2″), 78.46 (C-3″), 78.36 (C-5″), 78.19 (C-2‴), 75.45 (C-4‴), 71.34 (C-4″), 65.91 (C-5‴), 62.49 (C-6″), 56.75 (O<u>CH$_3$</u>). ESI-MS *m/z* 595.4 $[M + H]^+$, $C_{27}H_{30}O_{15}$. IR (KBr) $\nu_{max}$: 3445 (O-H), 2895 (C-H), 1640 (C=O), 1585, 1426 (C=C), 1175, 1050 (C-O) cm$^{-1}$.

## Results and discussion

Compound **1** was assigned to have a molecular formula of $C_{21}H_{20}O_{10}$ by a combination of its NMR data and ESI-MS *pseudo*-molecular ion peak at *m*/z 433.3 $[M+H]^+$. The presence of intramolecularly hydrogen-bonded proton signal [$\delta_H$ 12.96 (1H, s)] suggested that this hydroxyl group was at C-5. The $^1$H-NMR spectrum showed signals due to a *para*-disubstituted B-ring [$\delta_H$ 7.96 (2H, d, *J*=9.0 Hz, H-2′, H-6′), 6.94 (2H, d, *J*=9.0 Hz, H-3′, H-5′)], a *meta*-

disubstituted A-ring [$\delta_H$ 6.83 (1H, d, *J*=1.5 Hz, H-8), 6.45 (1H, d, *J*=1.5 Hz, H-6)], and a one-proton singlet of the C-ring [$\delta_H$ 6.86 (1H, s, H-3)] on the flavone skeleton. In addition, signals of a glucopyranosyl unit [$\delta_H$ 5.06 (1H, d, *J*=7.0 Hz, H-1″), and 3.72-3.17 (6H, m, H-2″, H-3″, H-4″, H-5″, H-6″)] were also observed. The large coupling constant (*J*=7.0 Hz) of the anomeric proton indicated the *β*-configuration of the glucopyranosyl moiety [9]. The $^{13}$C-NMR and heteronuclear single quantum coherence (HSQC) spectra revealed 15 carbon signals due to a flavone skeleton comprising one carbonyl carbon, seven sp$^2$ methine, and seven sp$^2$ quaternary carbons; in addition to six carbon signals due to a glucopyranosyl moiety. The heteronuclear multiple bond correlation (HMBC) from the anomeric proton H-1″ ($\delta_H$ 5.06) and two protons H-6 ($\delta_H$ 6.45) and H-8 ($\delta_H$ 6.83) to the carbon of A-ring at $\delta_C$ 162.96 confirmed that the glucosyl unit was at C-7. From the above spectral data, the structure of **1** was determined to be apigenin 7-*O*-*β*-glucoside. The $^{13}$C-NMR data of **1** were in good agreement with those of apigenin 7-*O*-*β*-glucoside in the literature [10].

Compound **2** was isolated as a yellow solid. Its molecular formula was established as $C_{22}H_{22}O_{11}$ on the basis of an ion peak $[M + H]^+$ at *m/z* 463.4 in ESI-MS. $^1$H- and $^{13}$C-NMR spectral data of compound **2** were similar with those of **1** except for the presence of three aromatic protons of an ABX spin system of ring B at $\delta_H$ 7.60 (1H, dd, *J*=8.5, 1.5 Hz, H-6′), 7.59 (1H, d, *J*=1.5 Hz, H-2′), and 6.95 (1H, d, *J*=8.5 Hz, H-5′); and an additional methoxyl group at $\delta_H$ 3.89 (3H, s). The position of the methoxyl group was at C-3′ based on the nuclear Overhauser effect spectroscopy (NOESY) correlations of H-2′ ($\delta_H$ 7.59) with 3′-OCH$_3$ ($\delta_H$ 3.89) and the HMBC correlations between the protons of 3′-OCH$_3$ ($\delta_H$ 3.89), H-2′ ($\delta_H$ 7.59), H-6′ ($\delta_H$ 7.60), and carbon C-3′ ($\delta_C$ 148.04). Moreover, the carbon chemical shifts of C-1′-C-6′ in **2** were very similar to those reported for chrysoeriol [11]. On the basis of the above evidence, the structure of **2** was elucidated to be chrysoeriol 7-*O*-*β*-glucoside [12].

The $^{13}$C-NMR and HSQC spectra of **3** confirmed the presence of 27 carbons that were attributed to 15 carbons of a flavone skeleton and 12 carbons of two sugar residues. The $^1$H-NMR spectrum showed resonance for two *meta*-coupled aromatic protons at $\delta_H$ 6.81 (1H, d, *J*=2.0 Hz, H-8) and 6.48 (1H, d, *J*=2.0 Hz, H-6); four *ortho*-coupled aromatic protons of an AA′BB′ spin system at $\delta_H$ 7.90 (2H, d, *J*=9.0 Hz, H-2′, H-6′) and 6.96 (2H, d, *J*=9.0 Hz, H-3′, H-5′); and a one-proton singlet at $\delta_H$ 6.68 (1H, s, H-3). Thus, the flavonoid moiety of **3** was determined as apigenin. In addition, a series of sugar signals at *δ* 3.97-3.41, along with two signals at $\delta_H$ 5.31 (1H, d, *J*=2.0 Hz, H-1‴) and 5.22 (1H, d, *J*=8.0 Hz, H-1″) corresponding to anomeric protons of two sugar residues, were also observed in the $^1$H-NMR spectrum. The coupling constants (*J*=8.0 Hz and 2.0 Hz) of the anomeric protons indicated the *β*- and *α*-configurations for glucopyranosyl and rhamnopyranosyl, respectively [9, 13]. The HMBC correlation from the anomeric proton H-1″ of glucose to C-7 ($\delta_C$ 164.43), H-1‴ ($\delta_H$ 5.31) with the carbon C-2″ ($\delta_C$ 79.11), and H-2″ ($\delta_H$ 3.74) with the carbon C-1‴ ($\delta_C$ 102.54) indicated 7-*O*-glycosidation and the interglycosidic linkage in **3** as -*O*-*α*-rhamnopyranosyl(1→2)-*O*-*β*-glucopyranoside. On the basis of the above evidence, the structure of **3** was assigned to be rhoifolin [14].

Compound **4** was obtained as a yellow solid. Its molecular formula was established as $C_{27}H_{30}O_{15}$ on the basis of an ion peak $[M+H]^+$ at *m/z* 595.4 in ESI-MS. The $^1$H- and $^{13}$C-NMR spectra of **4** were similar to those of **2** except for the appearance of an additional *α*-arabinofuranosyl moiety [$\delta_H$ 5.48 (1H, d, *J*=1.5 Hz, H-1‴); $\delta_C$ 110.94 (C-1‴), 80.71 (C-3‴), 78.19 (C-2‴), 75.45 (C-4‴), 65.91 (C-5‴)]. The *α*-arabinofuranosyl moiety was attached to C-2″ of glucopyranosyl based on the HMBC correlations between the anomeric proton H-1‴ ($\delta_H$ 5.48) and carbon C-2″ ($\delta_C$ 78.82) and between proton H-2″ ($\delta_H$ 3.69) and carbon C-1‴ ($\delta_C$ 110.94). Based on the evidence above and in comparison with those reported in the literature [15], compound **4** was determined to be 3′-methoxyapiin (Fig. 1).

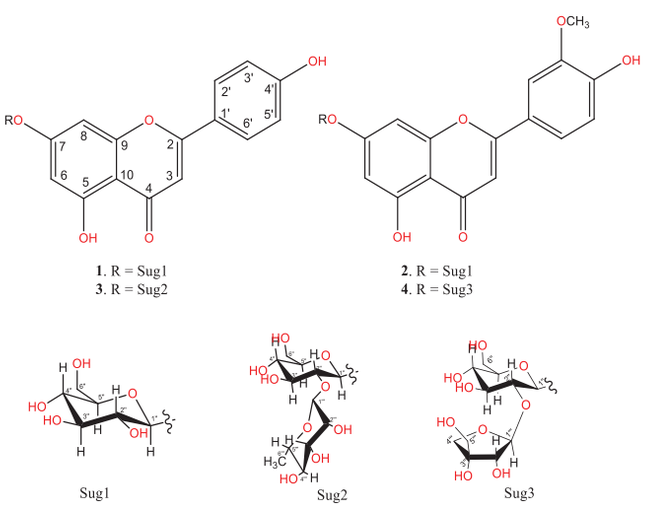


**Fig. 1. Structure of compounds 1-4.**

## Conclusions

From the n-butanol extract of the whole plant *U. crinita*, using column chromatography, four flavone glycosides including apigenin 7-*O*-β-glucoside (**1**), chrysoeriol 7-*O*-β-glucoside (**2**), rhoifolin (**3**), and 3′-methoxyapiin (**4**) were isolated. The structures of compounds **1-4** were determined by spectroscopic methods and comparison with published data. This is the first report of these compounds from the genus *Uraria*.

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## COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

## REFERENCES

[1] P. H. Ho (2000), "Cay co Vietnam [An illustrated flora of Vietnam]", Youth Publishing House, Ho Chi Minh city, Vietnamese, **1**, pp. 930-933.

[2] G.C. Yen, H.H. Lai, H.H. Chou (2001), "Nitric oxide-scavenging and antioxidant effects of *Uraria crinita* root", *Food Chemistry*, **74**, pp.471-478.

[3] Y.W. Mao, R.D. Lin, H.C. Hung, M.H. Lee (2014), "Stimulation of osteogenic activity in human osteoblast cells by edible *Uraria crinita*", *Journal of Agricultural and Food Chemistry,* **62**, pp.5581-5588.

[4] N. Morita, M. Arisawa, M. Nagase, H.Y. Hsu, Y.P. Chen (1977), "Studies on the constituents of Formosan Leguminosae. II. The constituents in the leaves of *Uraria crinita* Desv.", *Journal of the Pharmaceutical Society Japan*, **97**, pp.701-703.

[5] Y. Wang, X. Zhang, L. Gong, H. Ruan, H. Pi, Y. Zhang (2009), "Studies on chemical constituents in roots of *Uraria crinita*", *Chinese Pharmaceutical Journal*, **44**, pp.1217-1220.

[6] M. Okawa, R. Akahoshi, K. Kawasaki, D. Nakano, R. Tsuchihashi, J. Kinjo, T. Nohara (2019), "Two new triterpene glycosides in the roots of *Uraria crinita*", *Chemical and Pharmaceutical Bulletin*, **67**, pp.159-162.

[7]. D.D. Thien, T.T. Thuy, N.T.H. Anh, L.Q. Thang, T.D. Dai, N.H. Sa, N.T. Tam (2019), "A new isoflavanone from *Uraria crinita*", *Natural Product Reseach*, DOI: 10.1080/14786419.2019.1667352, pp.1-7.

[8] D.D. Thien, B.H. Tai, T.D. Dai, N.H. Sa, T.T. Thuy, N.T.H. Anh, N.T. Tam (2020), "New phenolics from *Uraria crinita* (L.) DC.", *Natural Product Reseach*, DOI: 10.1080/14786419.2020.1860973, pp.1-8.

[9] S.K. Hyun, S.S. Kang, K.H. Son, H.Y. Chung, J.S. Choi (2005), "Biflavone glucosides from *Ginkgo biloba* yellow leaves", *Chemical and Pharmaceutical Bulletin*, **53**, pp.1200-1201.

[10] P.V. Giang, P.T. Son (2004), "Apigenin 7-*O*-*β*-glucoside from the leaves of *Acanthus integrifolius* T. Anders., Acanthaceae", *Vietnam Journal of Chemistry*, **42**, pp.496-498.

[11] H. Wagner, V. M. Chari (1976), "$^{13}$C-NMR-spektren natürlich vorkommender flavonoide", *Tetrahedron Letters*, **21**, pp.1799-1802.

[12] C.M.N. Ngaffo, R.S.V. Tchangna, A.T. Mbaveng, J. Kamga, F.M. Harvey, B.T. Ngadjui, C.G. Bochet, V. Kuete (2020), "Botanicals from the leaves of *Acacia sieberiana* had better cytotoxic effects than isolated phytochemicals towards MDR cancer cells lines", *Heliyon*, **6**, DOI: 10.1016/j.heliyon.2020.e05412.

[13] A.L. Piccinelli, A. Veneziano, S. Passi, F.D. Simone, L. Rastrelli (2007), "Flavonol glycosides from whole cottonseed by-product", *Food Chemistry*, **100**, pp.344-349.

[14] E.J. Lee, J.S. Kim, H.P. Kim, J.-H. Lee, S.S. Kang (2010), "Phenolic constituents from the flower buds of *Lonicera japonica* and their 5-lipoxygenase inhibitory activities", *Food Chemistry*, **120**, pp.134-139.

[15] V.K. Thu, N.T.K. Thoa, P.V. Kiem (2016), "Flavonoid glycosides from *Viscum album*", *Vietnam Journal of Chemistry*, **54**, pp.443-447.