Pergamon

Tetrahedron Letters 42 (2001) 9015–9017

TETRAHEDRON LETTERS

Novel indole-type glucosinolates from woad (*Isatis tinctoria* L.)

Antoine Fréchard,[a] Nicolas Fabre,[a,*] Christophe Péan,[b] Sabine Montaut,[a] Marie-Thérèse Fauvel,[a] Patrick Rollin[c] and Isabelle Fourasté[a]

[a]*Laboratoire Pharmacophores Rédox, Phytochime et Radiobiologie, EA 3030, Université Paul Sabatier, Faculté des Sciences Pharmaceutiques, 35 chemin des Maraîchers, F-31062 Toulouse cedex 4, France*

[b]*Institut de Recherches Servier, 11 rue des Moulineaux, F-92150 Suresnes, France*

[c]*Institut de Chimie Organique et Analytique, UMR 6005, Université d'Orléans, BP 6759, F-45067 Orléans cedex 2, France*

Received 3 October 2001; revised 25 October 2001; accepted 26 October 2001

Abstract—Four novel indole-type glucosinolates (**1**–**1′** and **2**–**2′**), together with six other known glucosinolates, were isolated from the seeds of *Isatis tinctoria* L. and their structures elucidated by spectroscopic analysis. Combining an aliphatic and an indole moiety, they represent an original family of glucosinolates, thus broadening the structural diversity of those plant metabolites.

Isatis tinctoria L. or woad (Brassicaceae) is, in Mediterranean countries, a common plant cultivated throughout centuries to produce the blue dye indigo. Nowadays, woad is also used in Chinese folk and modern medicine.[1] Previous studies have shown its high indole glucosinolate content.[2,3] Recently, these compounds and/or their enzymatic breakdown products have been intensively studied for their cancer chemoprotective attributes.[4,5] From a methanolic extract of woad seeds, new epimeric indole glucosinolates (**1**–**1′**, **2**–**2′**, Fig. 1), displaying an original substitution pattern, have been isolated together with six other known glucosinolates (**3** to **7**). The aglycon of **1**–**1′** and **2**–**2′** corresponds to the 2-hydroxybuten-3-yl glucosinolates (**3**–**3′**), commonly called progoitrin and epiprogoitrin, depending on the configuration at C-3. The hydroxyl group of either of the two epimers is esterified as a 2,3-dihydro-2-oxo-1*H*-indol-3-yl acetate (oxIAA) for **1**–**1′** or a 2,3-dihydro-3-hydroxy-2-oxo-1*H*-indol-3-yl acetate (dioxIAA) for **2**–**2′**. The parent oxIAA and dioxIAA have previously been isolated in rice bran.[6] These glucosinolates represent a novel aglycon skeleton bearing a new indole moiety. In other respects, they may be considered as novel plant growth factors metabolites connected with indoleacetic acids (IAA). The structures of the previously known glucosinolates **3**–**7** have been confirmed by comparison of their spectral data with literature.[7,8]

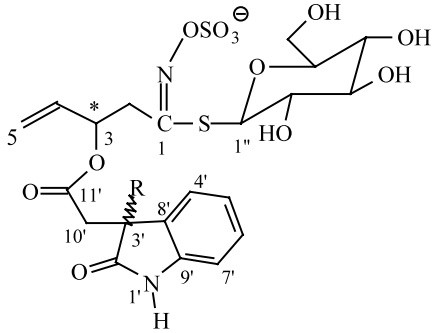
Keywords: oxindole; dioxindole glucosinolates; *Isatis tinctoria* L.; woad; Brassicaceae.

* Corresponding author. Tel.: +33 5 62256848; fax: +33 5 61554330; e-mail: nfabre@cict.fr

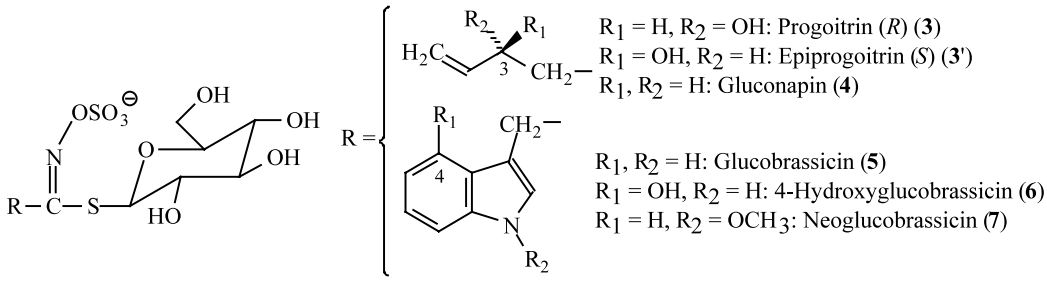
The epimeric mixtures of **1**–**1′** (100 mg) and **2**–**2′** (10 mg) as well as the other compounds were obtained as white amorphous powders using mild separative methods.[9] Pulverized dried seeds (800 g) were extracted twice with boiling MeOH (2 L) for 1 h. The combined filtrates were concentrated and the extract was then defatted by a liquid–liquid extraction using 2 L of $CHCl_3$–H_2O (1:1). The aqueous fraction was then chromatographed on a silica gel column with a gradient of $(CH_3)_2CO$–MeOH. The different fractions were monitored by reverse-phase (C-18) HPLC using a MeOH (containing 5 mM tetraheptylammonium bromide as ion-pairing agent)/phosphate buffer (pH 7.00; 0.01 M) (3:2) as mobile phase. The glucosinolate-containing fractions, eluted with 90% of $(CH_3)_2CO$, were separated on a C-18 silica gel cartridge eluted with a H_2O–MeOH gradient and the glucosinolates obtained were finally purified on a Sephadex® LH-20 column with MeOH as solvent.

The UV spectra showed absorption maxima at 206, 228 and 273 nm ($\log \varepsilon$ 4.29, 3.90 and 3.23, respectively) for **1**–**1′** and 210, 235, 247 and 289 nm ($\log \varepsilon$ 3.85, 3.38, 3.17 and 2.70, respectively) for **2**–**2′**—values that are suggestive of oxindole and dioxindole chromophores.[6] Their molecular formula were determined to be $C_{21}H_{25}N_2O_{12}S_2$ for **1**–**1′** and $C_{21}H_{25}N_2O_{13}S_2$ for **2**–**2′** by negative-ion HRFABMS (m/z 561.085 $[M]^-$ and m/z 577.080 $[M]^-$, respectively). It is worth taking notice that in the positive-ion mode, *pseudo*-molecular ions at m/z 607 for **1**–**1′** and m/z 623 for **2**–**2′** were obtained as base peaks, corresponding to $[MNa+Na]^+$ ions. The ^1H

0040-4039/01/$ - see front matter
PII: S0040-4039(01)02015-9



R = H: Glucoisatisin (*R*) and Epiglucoisatisin (*S*) (**1-1'**)
R = OH: 3'-Hydroxyglucoisatisin (*R*) and 3'-Hydroxyepiglucoisatisin (*S*) (**2-2'**)



R = R_1, R_2 = H: Progoitrin (*R*) (**3**)
R_1 = H, R_2 = OH: Epiprogoitrin (*S*) (**3'**)
R_1, R_2 = H: Gluconapin (**4**)
R_1, R_2 = H: Glucobrassicin (**5**)
R_1 = OH, R_2 = H: 4-Hydroxyglucobrassicin (**6**)
R_1 = H, R_2 = OCH_3: Neoglucobrassicin (**7**)

Figure 1. Glucosinolates isolated from woad.

Table 1. NMR data for **1**–**1′** and **2**–**2′** (^{1}H NMR 500.13 MHz; ^{13}C NMR 125.7 MHz; DMSO-d_6; data in ppm (*J* in Hz))

Position	**1**–**1′**		**2**–**2′**	
	^{13}C	^{1}H ppm (*J*, Hz)	^{13}C	^{1}H ppm (*J*, Hz)
Progoitrin moiety				
1	152.2	–	153.0	–
2	36.2	*R* or *S* 2. 60 m–2.71 m	36.9	*R* or *S* 2.58 m–2.65 m
		R or *S* 2.76 m–2.84 m		*R* or *S*: 2.48 m–2.55 m
3	72.1	5.45 *R* or *S* quint. (6.0)	72.6	5.20 m
4	135.2	5.78 *R* or *S* ddd (17.1–10.6–6.0)	135.6	5.55 *R* or *S* ddd (17.1–10.6–6.0)
		5.82 *R* or *S* ddd (17.1–10.6–6.4)		5.65 *R* or *S* ddd (17.1–10.6–6.0)
5	116.7	5.10 dt (10.6–1.1)	117.5	4.98 dt (10.6–1.1)
		5.20 dt (17.1–1.1)		5.05 dt (17.1–1.1)
Oxindole and dioxindole moieties				
1′	–	10.40 s	–	10.28 s
2′	178.7	–	178.7	–
3′	42.0	3.68 m	73.9	–
4′	123.7	7.24 d (7.8)	125.0	7.35 d (7.1)
5′	121.8	6.93 t (7.8)	122.4	6.96 t (7.4)
6′	128.0	7.16 t (7.8)	130.1	7.21 t (7.6)
7′	109.5	6.81 d (7.8)	110.5	6.81 d (7.5)
8′	129.0	–	131.6	–
9′	142.8	–	143.5	–
10′	34.0	2.92 m	42.9	3.01 m
		3.04 m		3.05 m
11′	170.0	–	168.5	–
1-Thio-β-D-*gluco* moiety				
1″	82.0	4.87 (d 9.9)	82.7	4.80 d (9.7)
2″	72.9	3.04–3.22 m	73.7	3.08–3.28 m
3″	78.1	3.04–3.22 m	78.9	3.08–3.28 m
4″	69.7	3.04–3.22 m	71.5	3.08–3.28 m
5″	81.0	3.04–3.22 m	81.6	3.08–3.28 m
6″	60.8	3.42 m–3.64 dd (12.1–2.0)	60.5	3.45 m–3.65 m

Note that for the progoitrin, oxindole and dioxindole moieties (^{1}H and ^{13}C NMR data of **1**–**1′** and **2**–**2′**), the chemical shifts values, mentioned in Table 1, were determined from the middle of the corresponding splitting signal.

and ^{13}C NMR data (Table 1) were indicative of glucosinolates,[7,8] i.e. the chemical shifts of the 1-thio-β-D-*gluco* moiety (position 1″: δ_C 82 ppm, δ_H 4.8 ppm ($J=9.9$ Hz); position 5″: δ_C 81 ppm) and the oximino carbon C-1 at δ_C 152 ppm. Concerning the aglycons, the aliphatic moiety was unambiguously assigned to a 2-hydroxybuten-3-yl group by comparison of its NMR data with the isolated compounds **3**–**3′** and literature.[7,8] The ^{13}C *J*-modulated NMR spectrum of **1**–**1′** revealed, for the oxIAA moiety, the presence of one carboxyl carbonyl (δ_C 170 ppm), one amide carbonyl (δ_C 178 ppm), one methylene (δ_C 34 ppm), a disubstituted phenyl group and one primary carbon at δ_C 42 ppm (C-3′). The only difference in the ^{13}C *J*-modulated NMR spectra of **1**–**1′** and **2**–**2′** resides in this latter carbon (C-3′) which was replaced by a quaternary one at δ_C 73.9 ppm in the ^{13}C NMR spectrum of **2**–**2′**. The difference of 16 u in the mass spectra of the two compounds and a chemical shift at δ_C 73.9 ppm for **2**–**2′** suggest the substitution of C-3′ by a hydroxyl group thus indicating a dioxIAA moiety. Moreover, the NMR data of these two oxIAA and dioxIAA moieties were in good agreement with previously published data.[6,10–12] The ^1H NMR spectra of **1**–**1′** and **2**–**2′** obtained in DMSO-d_6 showed the presence of exchangeable protons (singlets at δ_H 10.40 ppm for **1**–**1′** and δ_H 10.28 ppm for **2**–**2′**) characteristic of indole NH. Proton spin systems were determined by analysis of ^1H–^1H COSY spectra. Chemical shift assignments for carbon bound to hydrogen atoms were established on the basis of either of HSQC data. The structures of **1**–**1′** and **2**–**2′** were finally confirmed by careful analysis of HMBC and NOESY spectra (Fig. 2). Concerning the stereochemistry of chiral centers (C-3 and C-3′), the occurrence of epimeric mixtures of **1**–**1′** and **2**–**2′** (splitting of aglycons signals in the ^1H and ^{13}C NMR spectra) implying the C-3 carbon were clearly deduced from ^1H NMR and COSY spectra: particularly the two protons H-4 (*R* and *S*) and the two AB systems H-2 (*R* and *S*) which appeared well separated and presented equivalent integration. This was supported by the interpretation of the ^1H NMR spectrum of compounds **3**–**3′** that were obtained as a mixture of 75% of progoitrin (*R*) and 25% of epiprogoitrin (*S*). Unfortunately, it was not possible to unambiguously determine the configuration of carbon C-3′ given rotational freedom in the C-3′ side chain and the lack of significant NOEs. Therefore,

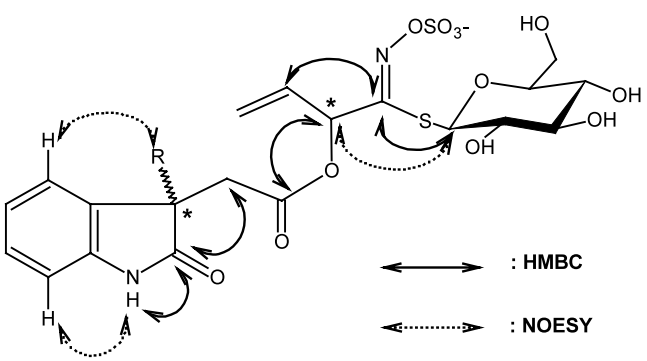


Figure 2. Significant correlations observed in the HMBC and NOESY spectra of **1**–**1′** (R = H) and **2**–**2′** (R = OH).

1–**1′** was determined as 1-thio-β-D-glucopyranose-1-[3-(2′,3′-dihydro-2′-oxo-1*H*-indol-3′-yl-acetate)-*N*-(sulfooxy)-4-pentanenimidate] and **2**–**2′** as 1-thio-β-D-glucopyranose-1-[3-(2′,3′-dihydro-3′-hydroxy-2′-oxo-1*H*-indol-3′-yl-acetate)-*N*-(sulfooxy)-4-pentanenimidate]. For the sake of simplification, we suggest to commonly call them glucoisatisin and 3′-hydroxyglucoisatisin, respectively.

Finally, it is worth mentioning that other oxIAA and dioxIAA derivatives have been isolated as products of the β-indolylacetic acid (IAA) metabolism from kernels of *Zea mays* L. and from rice bran.[10,12] Moreover, various works explained the IAA degradation pathway by oxidation in oxIAA and/or dioxIAA followed by a conjugation with amino acids and sugars.[13–15]

However, to date, the occurrence of other secondary plant metabolites esterified with dioxIAA has only been described for a flavonoid glycoside.[16]

Acknowledgements

The authors wish to thank Dr. Renato Iori (ISCI Bologna) for fruitful discussions.

References

1. Pinkas, M.; Peng, W.; Torck, M.; Trotin, F. *Plantes Médicinales Chinoises*; Maloine: Paris, 1996; p. 86.
2. Lockwood, G. B.; Belkhiri, A. *Plant Syst. Evol.* **1991**, *176*, 11–20.
3. Fahey, J. W.; Zalcmann, A. T.; Talalay, P. *Phytochemistry* **2001**, *56*, 5–51.
4. Stoewsand, G. S. *Food Chem. Toxicol.* **1995**, *33*, 537–543.
5. Staack, R.; Kingston, S.; Wallig, M. A.; Jeffery, E. H. *Toxicol. Appl. Pharm.* **1998**, *149*, 17–23.
6. Kinashi, H.; Suzuki, Y.; Takeuchi, S.; Kawarada, A. *Agric. Biol. Chem.* **1976**, *40*, 2465–2470.
7. Cox, I. J.; Hanley, A. B.; Belton, P. S.; Fenwick, G. R. *Carbohydr. Res.* **1984**, *132*, 323–329.
8. Prestera, T.; Fahey, J. W.; Holtzclaw, W. D.; Abeygunawardana, C.; Kachinski, J. L.; Talalay, P. *Anal. Biochem.* **1996**, *239*, 168–179.
9. Fabre, N.; Bon, M.; Moulis, C.; Fourasté, I.; Stanislas, E. *Phytochemistry* **1997**, *45*, 525–527.
10. Tateishi, K.; Shibata, H.; Matsushima, Y.; Iuima, T. *Agric. Biol. Chem.* **1987**, *51*, 3445–3447.
11. Tateishi, K.; Matsushima, Y.; Shibata, H. *Agric. Biol. Chem.* **1989**, *53*, 2545–2551.
12. Tateishi, K.; Yamashita, S. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 1870–1874.
13. Ostin, A.; Kowalyczk, M.; Bhalerao, R. P.; Sandberg, G. *Plant Physiol.* **1998**, *118*, 285–296.
14. Bialek, K.; Cohen, J. D. *Plant Physiol.* **1989**, *91*, 775–779.
15. Chamarro, J.; Ostin, A.; Sandberg, G. *Phytochemistry* **2001**, *57*, 179–187.
16. Hubner, G.; Wray, V.; Nahrstedt, A. *Planta Med.* **1999**, *65*, 636–642.