Proc. Indian Acad. Sci., Vol. 88 A, Part I, Number 5, October 1979, pp. 323-327,

Oroxindin—A new flavone glucuronide from *Oroxylum indicum* Vent.

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MS received 30 March 1979

Abstract. From the ethanol extract of the seeds of *Oroxylum indicum* a new flavone glucuronide named oroxindin has been isolated. Based on chemical as well as UV, IR, PMR and mass spectral data oroxindin has been constituted as 5-hydroxy-8-methoxy-7-0-β-D-glucopyranuronosyl flavone (wogonin-7-0-β-D-glucuronide).

Keywords. Flavone glucuronide; *Oroxylum indicum*; Bignoniaceae; wogonin-7-0-β-D-glucuronide; PMR spectrum of lactone acetate.

1. Introduction

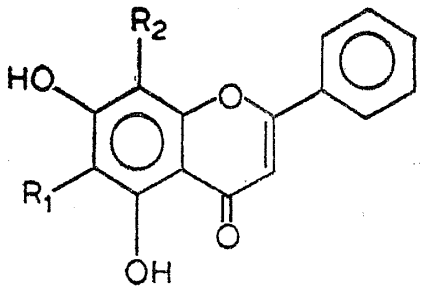
The flavonoids of *Oroxylum indicum* Vent. (family : Bignoniaceae) have been examined earlier; baicalein-6-glucoside (tetuin) is recorded from its seeds (Mehta and Mehta 1959) while the leaves contain baicalin, baicalein-6-0-glucuronide and scutellarin (Nair and Subramanian 1972a) and the bark has oroxylin-A and dihydrobaicalein in addition to the leaf flavones (Nair and Subramanian 1972b). Recently Vandor *et al* (1977) have synthesised baicalein-6-glucoside and found it to be different from tetuin and further considered that tetuin could not be a glycoside of baicalein at all. It was therefore desired to re-examine the seeds of *Oroxylum indicum* for flavonoids in order to establish the true structure of tetuin and the results are presented here. While we could not identify any baicalein glycoside in the seeds, they yielded a new flavone glucuronide which was characterised as 5-hydroxy-8-methoxy-7-0-β-D-glucopyranuronosyl flavone (wogonin-7-0-β-D-glucuronide) to which we propose the name oroxindin.

2. Results and discussion

The light yellow flavone glycoside isolated from the EtOAc extract of the alcoholic concentrate of the seeds after three crystallisations from MeOH had m.p. 210–11°, $[\alpha] = -50°$ (C_5H_5N), λ_{max} (nm) : 275, 340 (MeOH), 280, 388 (NaOMe), 275, 340 (NaOAc and NaOAc/H_3BO_3) and 280, 330sh, 348, 390sh ($AlCl_3$) and ν_{max} 3400 br (multiple OH), 1720 (carboxyl) and 1640 (bonded carbonyl) cm^{-1}. Its

PMR spectrum (DMSO-d6) exhibited signals at 11·4 (br *s*, 2H, COOH and 5–OH) 8·0 and 7·6 (pair of *m*, 5H, H–2′, 3′, 4′, 5′ and 6′), 6·9 (*s*, 1H, 3–H) 6·6 (*s*, 1H, 6–H), 4·0 (*s*, 3H, OCH_3) and 3·6 (br *s*, H of sugar/water). On acetylation it gave the lactone acetate, $C_{28}H_{24}O_{13}$, m.p. 189–90°, $[\alpha] = -96°$ ($CHCl_3$). The PMR spectrum ($CDCl_3$) of the acetate gave further evidence for the presence of a 5,7-dihydroxy-6/8-methoxy flavone-7-0-glucuronide lactone derivative (see experimental). Comparison of the PMR spectrum with those of apigenin-7-0-β-D-glucopyranuronoside-3,6-lactone acetate as well as the 3,6-lactone acetates of β-naphthol-β-D-glucopyranuronoside and furanuronoside (Wagner *et al* 1971) established that the glucuronic acid is present as the diacetate of its 3,6 lactone in the pyrano form. The mass spectrum of the acetate did not show the molecular ion but had the parent ion with m/e 526 indicating the facile loss of a $CH_2{=}CO$ from the molecular ion.

The glycoside had high R_f in water with marked decrease in 5% HOAc (typical of glycuronides) (Nair and Subramanian 1974) and was resistant to mild acid hydrolysis (1N HCl, 1 hr). On refluxing with 2N HCl for 3 hr as well as when treated with the enzyme β-glucuronidase, it underwent hydrolysis to yield an aglycone and D-glucuronic acid in equimolar ratio.



I

a — R_1 = H, R_2 = OMe : Wogonin

b — R_1 = OMe, R_2 = H : Oroxylin-A

The aglycone, $C_{16}H_{12}O_5$ (M.W. 284), m.p. 204–5°, was purple under UV and UV/NH_3, gave a stable yellow colour with alkali and had λ_{max} in MeOH (275, 340 nm) and with diagnostic reagents characteristic of 5,7-dihydroxy-6/8-methoxy flavone (Mabry *et al* 1970). The fragmentation pattern of the MS of the aglycone (M^+, 284, 65%; $M^+ - CH_3$, 269, 100%; no detectable $M^+ - 1$ or $M^+ - 18$) indicated that the OMe is at C–8 and not at C–6 (Goudard *et al* 1978) pointing to the identity of the flavone as 5,7-dihydroxy-8-methoxy flavone (wogonin) (Ia). The remote possibility of the aglycone to be 5,7-dihydroxy-6-methoxy flavone (oroxylin-A) (Ib) was ruled out by direct comparison (especially co-PC in aqueous solvents) of our sample and its demethylation product (norwogonin) with oroxylin-A and baicalein respectively. Unequivocal differentiation of the isomers, wogonin and oroxylin-A by physical constants has been reported to be difficult (Joshi and Gawad 1977). Direct comparison of the mass spectrum m.m.p. R_f on SiO_2 treated with 0·5N oxalic acid and IR of our sample with those of authentic oroxylin-A and wogonin confirmed the identity of the aglycone as wogonin and thus the glycoside was finally identified as wogonin glucuronide, a new glycoside designated as oroxindin.

The identical λ_{max} of the glycoside and its aglycone as well as the absence of any shift in the NaOAc spectrum of the glycoside established the involvement of 7–OH in glycosidation. The enzyme hydrolysis showed that the sugar was

β-linked while the PMR spectrum revealed its pyranoside structure. Thus, oroxindin has been constituted as 5-hydroxy-8-methoxy-7-0-β-D-glucopyranuronosyl flavone (II) and its triacetate as 5,2″,4″-tri-0-acetyl oroxindin-3″,5″-lactone (III).

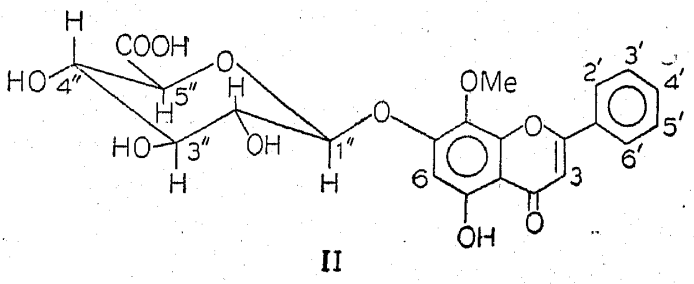
3. Experimental

Melting points were determined by open capillary method and are uncorrected. UV spectra were recorded on Beckmann DK2A spectrometer, IR spectrum (KBr) by Perkin–Elmer Infracord spectrophotometer and 90 MHz FT–PMR spectra by Bruker WH 90 spectrometer using TMS as internal standard; values are expressed in δ, ppm. Mass spectra were run on Atlas CH–7 instrument and rotation measured by B and S polarimeter using the *D* line of sodium at 28° C.

3·1. *Isolation of oroxindin*

The fresh seeds (800 g) from mature fruits of *Oroxylum indicum*, collected from Kerala State, were extracted with boiling 80% EtOH (2 × 5 litre) and the extract concentrated under vacuum to about 400 ml. This was extracted with C_6H_6, Et_2O and EtOAc. The solid from the EtOAc concentrate (200 mg) when crystallised thrice from MeOH yielded light yellow needles (II; 110 mg), m.p. 210–11°, [α] −50° (C_5H_5N). For UV and PMR spectra see discussion. IR spectrum (KBr) 3400 br, 2925, 1720, 1640, 1600, 1480, 1250, 1100 br, 1040, 940, 860, 780 and 695 cm^{-1}. (Found: C, 57·2; H, 4·6. Calc. for $C_{22}H_{20}O_{11}$: C, 57·4, H, 4·3%).

The compound (40 mg) on treatment with Ac_2O (1 ml) and pyridine (5 drops) gave the triacetate (III; 30 mg), m.p. 189–90° (EtOAc-petrol), [α] −96° ($CHCl_3$); δ ($CDCl_3$) 7·9 and 7·55 (pair of *m*, 5H, H–2′, 3′, 4′, 5′ and 6′) 6·9 (*s*, 1H, 3–H), 6·66 (*s*, 1H, 6–H) 5·74 (*s*, 1H, 1″–H) 5·52 (*d*, J = 4Hz, 1H, 2″–H) 5·22 (*t*, J = 4Hz, 1H, 3″–H) 5·0 (*t*, J = 4Hz, 1H, 4″–H) 4·4 (*d*, J = 4Hz, 1H, 5″-H) 4·02 (*s*, 3H, 8–OCH_3) 2·44 (*s*, 3H, 5–$OCOCH_3$) 2·24 and 2·18 (*s* each, 3H each, 4″ and 2″–$OCOCH_3$) m/e 526 (lactone acetate–CH_2=CO), 326, 284, 269, 255, 243, 167, 149, 111 and 97. (Found : C, 58·8; H, 4·3. Calc. for $C_{28}H_{24}O_{13}$: C, 59·1, H, 4·2%).



II

Oroxindin

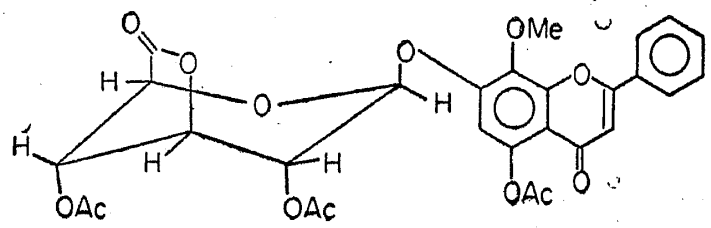
3·2. *Acid hydrolysis of oroxindin*

The glycoside (50 mg) was refluxed with 2N HCl in 50% MeOH medium on a waterbath for 3 hr and the aglycone obtained by usual working up was recrystallised from acetone to yield pale yellow needles of wogonin (Ia; 25 mg), m.p. 204–5° (lit. m.p. 203°) (Harris 1955) λ_{max} 275, 340 (MeOH), 283, 375 (NaOMe), 283,

355 (NaOAc), 275, 340 ($NaOAc/H_3BO_3$), 292, 333 sh, 395 ($AlCl_3$); m/e 284 (M^+, $C_{16}H_{12}O_5$, 65%) 269 (M^+-CH_3, 100), 255 (M^+-CHO, 6) 241 (M^+-CH_3CO, 31) and 167 (A–ring f ɛgment–CH_3, 11) (Found: C, 67·5; H, 4·4. Calc. for $C_{16}H_{12}O_5$: C, 67·6; H, 4·2%) (m/e for oroxylin: 284 (M^+, 100%), 283 (M^+-1, 6), 269 (M^+-CH_3, 75), 266 (M^+-H_2O, 43), 254 (283–CHO, 57), 240 (283–$CO-CH_3$, 85) and 167 (A-ring fragment–CH_3 71%).

3·3. *Enzyme (glucuronidase) hydrolysis of oroxindin*

Oroxindin (5 mg) was dissolved in 0·05M acetic acid/sodium hydroxide buffer (pH, 5·2) (5 ml), mixed with β-glucuronidase (Sigma Chemical Company, USA) (2 mg) and kept in an incubator at 38° for 12 hr. The aglycone was extracted with ether and compared with wogonin obtained by acid hydrolysis. The sugar portion in both cases was found to be D-glucuronic acid by co-chromatography with an authentic sample.



III

2″, 4″, 5–triacetyl oroxindin–3″, 5″–lactone

3·4. *Demethylation of wogonin*

Wogonin (10 mg) was dissolved in hot Ac_2O (1 ml), cooled and to the solution added HI (BDH, microanalytical grade, 1 ml) drop wise, the mixture refluxed for 1 hr, cooled and poured into cold sodium metabisulphite solution. The yellow solid separated was crystallised from acetone to yield norwogonin, m.p. 235–36° (lit. m.p. 257–9°; 227–8°) (Harris 1955). λ_{max} 274, 324 (MeOH), 254, 365 (NaOMe), 268, 350 (NaOAc), 265, 338 ($NaOAc/H_3BO_3$) and 273, 283, 373 ($AlCl_3$), m/e 270 (M^+, $C_{15}H_{10}O_5$, 100%), 168 (trihydroxy A-ring fragment) 105 (B-ring with CO) and 77 (side phenyl).

3·5. *Paper chromatography of oroxindin, wogonin and norwogonin (Oroxylin-A and baicalein included for comparison)*

Chromatography was carried out using Whatman No. 1 paper, by the ascending technique and R_f determined at 28 ± 1° C after a solvent flow of approximately 20 cm using the following developing solvents: water, 15% HOAc, 30% HOAc, 50% HOAc, BAW, Phenol and Forestal. $R_f \times 100$ is given in the order of the above solvents (— indicates no movement/trailing)

Oroxindin :	69, 43, 72, 83, 77, 64 and 89
Wogonin :	—, 10, 48, 76, 96, 96 and 90
Norwogonin:	—, 9, 43, 63, 94, 86 and 77
Oroxylin–A:	—, —, —, 55, 95, 97 and 89
Baicalein:	5, 17, 44, 63, 93, 88 and 76.

Acknowledgements

We thank Prof. M Takido for an authentic sample of wogonin, Dr S Selvavinayakam and his colleagues for the spectral and analytical data, the Director, Jawaharlal Institute, for encouragement and the UGC, New Delhi, for financial assistance.

References

Goudard M, Favre-Bonvin J, Lebreton P and Chopin J 1978 *Phytochemistry* **17** 145

Harris G 1955 *Dictionary of organic compounds* 4th edition (London: Eyre and Spottiswoode)

Joshi B S and Gawad D H 1977 *Proc. Indian Acad. Sci.* **A86** 41

Mabry T J, Markham K R and Thomas M B 1970 *Systematic identification of flavonoids* (Berlin: Springer-Verlag)

Mehta C R and Mehta T P 1959 *J. Indian Chem. Soc.* **36** 468

Nair A G R and Subramanian S S 1972a *Phytochemistry* **11** 439

Nair A G R and Subramanian S S 1972b *Curr. Sci.* **41** 62

Nair A G R and Subramanian S S 1974 *Bull. JIPMER Clin. Soc.* **10** 126

Vandor G M, Farkas L and Nogradi M 1977 *Flavonoids and bioflavonoids, current research Trends* (New York: Elsevier Scientific Publishing Co.)

Wagner H, Danninger H, Iyengar M A, Seligmann O, Farkas L, Nair A G R and Subramanian S S 1971 *Chem. Ber.* **104** 2681