ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLV, nr 4
1976

# Quantitative daily changes of flavonol glycosides in the leaves of *Betula humilis* Schrk.

LUCYNA PAWŁOWSKA

Department of Plant Variability, Institute of Botany, Polish Academy of Sciences

(Received: January 22, 1976)

Abstract

Rutin and hyperosid were isolated from fresh leaves of *Betula humilis*. The amount of flavonol glycosides increases to the maximum quantity between 9—10 a.m. and 1—2 p.m. in the period from July to September.

## INTRODUCTION

The influence of light on the biosynthesis of particular groups of flavonoid compounds was found out by McClure (1970). The relationships between the quantity of isolated flavonoid compounds and the colour of light and length of irradiation were studied by Smith et al. (1970a, 1970b) and Harper et al. (1970).

In the course of the earlier chemotaxonomic studies on various species of the genus *Betula* (Pawłowska n.publ.) the present author noticed the differences in the amounts of flavonol glycosides occuring in the leaves of the specimens growing under natural conditions during the daytime. These differences depend on the time of day when the material had been collected. This observation encouraged the author to carry out some further, more detailed investigations on this problem.

In the leaves of *Betula humilis* occur only two flavonol glycosides: rutin (quercetin 3-glucorhamnoside) as the main flavonoid compound, and hyperosid (quercetin 3-galactoside), (Hörhammer et al. 1953, Hänsel, Hörhammer 1954). Therefore this material has been chosen for the present studies.

## EXPERIMENTAL

### Material

The studies were carried out on fresh leaves of one specimen of *Betula humilis* growing in the Botanic Garden in Cracow on a much insolated site, slightly shaded from the east. Ten gram of mature leaves were collected at a time. The material was collected on: July 4. 1973 and September 1. 1973, from 8.00 a.m. to 6.00 p.m. every hour, duringand August 5 to 7, 1974 from 8.00 a.m. to 3.00 p.m. every hour, during the peaks every quarter of an hour.

It should be emphasized that the summer of the year 1974 was characterized by a great amount of rain-fall, and that there were very few sunny days.

### Methods

a. Extraction procedure

The material collected on dry ice was homogenized with petroleum ether and kept for 24 hours et the temperature approximately 0°C. On the following day it was centrifuged and washed with petroleum ether until the colouration disappeared in the ether phase. Next, 200 ml of methanol were added to the remains and the material was then kept in the refrigerator for 24 hours at the temperature approximately 0°C. After centrifuging the extract was repeated until a colourless filtrate was obtained.

b. Purification of the fractions of rutin and hyperosid

The combined methanol extracts of each sample were evaporated to the volume of 50 ml, and after adding chloroform in an equal volume it was thoroughly shaken with methanol and left for about 10 to 15 minutes. The methanol-aquatic phase was subjected to preparatory paper chromatography on Whatman 3 and developed in the solvent system: ethyl acetate — formic acid — water (10:2:3 v/v). Rutin appeared on the chromatograms as a very intensive yellow band with $R_f$ 0,37—0,39, and hyperosid as a band with a weak lemon-yellowish with $R_f$ 0,50—0,53. The brown bands below $R_f$ 0,25 and the greenish-yellow ones exceeding $R_f$ 0,60 were eliminated. The fractions from the chromatograms were extracted with methanol: rutin from the bands with $R_f$ 0,33—0,45 and hyperosid from the bands with $R_f$ 0,48—0,55. The volumes of the extracts were completed as follows: rutin up to 200 ml, and hyperosid up to 100 ml. Each extract was subjected to chromatography paper on What-

man 1 at the solution in the solvent systems of n-butanol — acetic acid — water (4:1:5 v/v) and 30% acetic acid, and they were compared with the standards.

c. Determination of the amounts of rutin and hyperosid

In each of the extracts, the absorption was estimated for rutin at 258 nm, and hyperosid at 257 nm using Beckman spectrophotometer Model 25. The readings of the amounts of the compounds under investigation were taken from the standard curves.

## RESULTS AND DISCUSSION

Rutin and hyperosid occurring in the leaves of *Betula humilis* (Table 1) show parallel changes (Fig. 1) in during daytime. The course of these changes depends on the date of collection of the material presented in Figure 2. for the rutin. As the amount of rutin is over twenty times higher then that of hyperosid a scheme presenting the changes in the former illustrates the process more distinctly. The first peak is marked in the morning between 9—10 o'clock; afterwards the amount of these two glycosides decreases, and then conspicuosly rises

Table 1

Characteristic of rutin and hyperosid from fresh leaves *Betula humilis*

| Compound | $R_f$ measurement solvent system | | | Absorption in methanol (nm) | | | |
|---|---|---|---|---|---|---|---|
| | A | B | C | | | | |
| Fraction a | 0.38 | 0.45 | 0.75 | 258 | 268 | 300 | 358 |
| Fraction b | 0.52 | 0.58 | 0.56 | 257 | 268 | 300 | 360 |
| Standards | | | | | | | |
| Rutin | 0.39 | 0.42 | 0.75 | 258 | 267 | 299 | 358 |
| Hyperosid | 0.51 | 0.56 | 0.55 | 257 | 269 | 301 | 362 |

Solvent system
A EtOAc-$HCO_2H$-$H_2O$ (10:2:3 v/v)
B n-BuOH-$H_2O$-HOAc (4:5:1 v/v)
C 30% HOAc

again attaining a peak equal to the previous one, or slightly higher, in the early afternoon hours between 1 and 2 p.m. From then on a gradual fall in the amount of these two glycosides has been observed, and in the late afternoon hours it shows slightly higher values then the initial ones.

The studies on the quantitative changes occurring in the flavonol glycosides in *Betula humilis* leaves point to a slight shifting of the peaks of amounts of the compounds investigated during the daytime, depending on the date of collection of the material (Fig. 2).

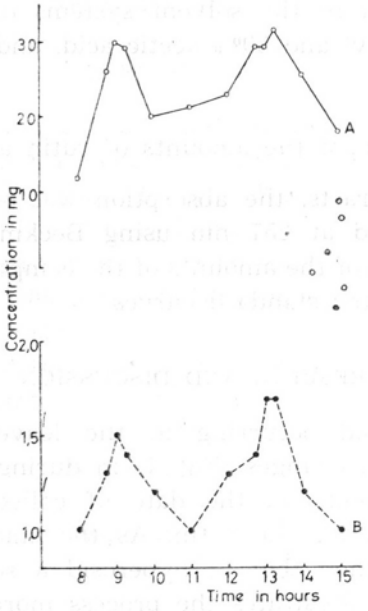


Fig. 1. Quantitative daily changes of rutin and hyperosid on 5.VIII.1974. A — rutin; B — hyperosid

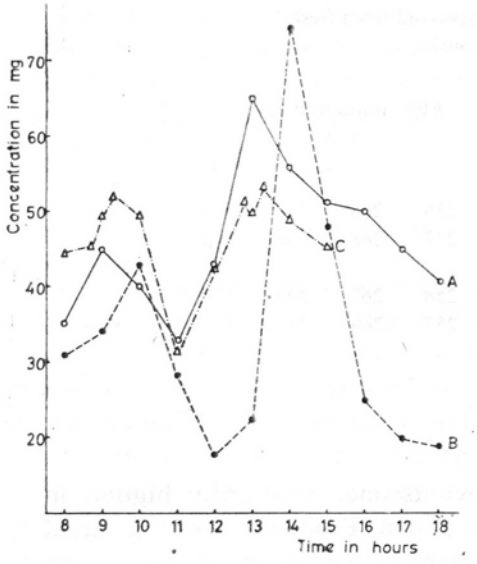


Fig. 2. Quantitative daily changes of rutin. A — 4.VII.1973; B — 1.IX.1973; C — 7.VIII.1974

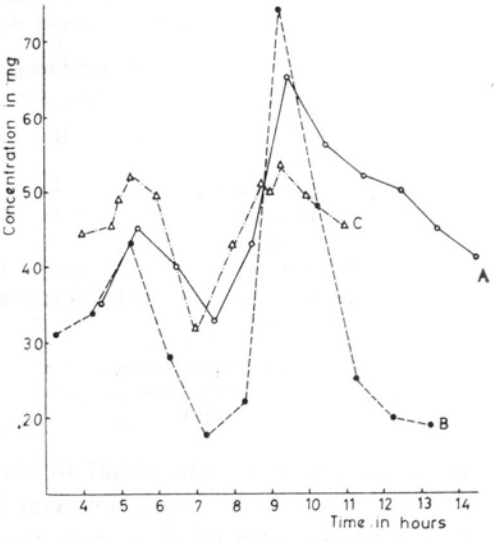


Fig. 3. Quantitative changes of rutin depending on the length of irradiation. The sunrise was accepted as the initial time of irradiation, A — 3.20 h (4.VII.1973); B — 4.46 h (1.IX.1973); C — 4.02 h (7.VIII.1974)

If the course of the changes in the compounds studied is compared with the changes in temperature at the same hours, no correlation between these values con be established, because at the time between 8 a.m. and 3 p.m. there only occurs a rise in temperature by 2° to 7°C. with an oscillation up to 0,5°C. However, one can observe a dependence of the amount of the compounds studied (at the maximum) on the atmospheric conditions prevailing on the day of collection of the material (Table 2).

Table 2

Isolated amounts of rutin and hyperosid at the peaks on particular days of collection of the material

| Date of collection | Weather conditions | Amount of rutin | | Amount of hyperosid | |
|---|---|---|---|---|---|
| | | first peak | second peak | first peak | second peak |
| 4.VII.1973 | Frequent intervals of clear weather | 45.0 | 65.0 | 1.60 | 1.86 |
| 1.IX.1973 | Sunshine with heat | 43.0 | 74.0 | 2.20 | 2.80 |
| 5.VIII.1974 | Cloudiness with clear | 30.0 | 31.6 | 1.50 | 1.70 |
| 6.VIII.1974 | Permanent cloudiness | 22.0 | 22.8 | 0.67 | 0.70 |
| 7.VIII.1974 | Sunshine | 42.4 | 52.8 | 2.40 | 2.90 |

All values are formulated in mg/10 g of fresh leaves.

If we accept the sunrise at the time when irradiation being under natural conditions (Fig. 3) it appears that the first peak of the amount of the compounds studied occurs after 5—6 hours, and the second after 9—10 hours. Studying the dependence of the amount of the flavonol glycosides (kaempferol 3-p-coumaroyltriglucoside and quercetin 3-p--coumaroyltriglucoside) on the time of irradiation, Smith et al. (1970), established that the maximum quantities of these comopunds undergo a biosynthesis after 5—6 hours and then after 10—12 hours of irradiation of the etiolated buds seedlings of *Pisum sativum*. Thus, the results obtained by the present author are similar to those refered above.

The course of the changes described above is most probably related with changes in activity of PAL (L-phenylalanine ammonia-lyase), the role of which as a photo-regulator has been proved by a number of various plants (Durst, Mohr 1966; Zucker 1969, 1970; Maier, Hasegawa 1970; Hahlbrock et al. 1971).

Acknowledgement

I wish to thank Dr Katarzyna Dziewanowska and Prof. dr Stanisław Lewak for their help in preparizing the present paper.

## REFERENCES

Durst F., Mohr H. 1966. Phytochrome — mediated induction of enzyme synthesis in mustard seedlings (*Sinapsis alba* L.). Naturwissenschaften 53: 531—532.

Hahlbrock K., Sutter A., Wellmann E., Ortman R., Grisebach H. 1971. Relationship between organ development and activity of enzymes involved in flavone glycoside biosynthesis in young parsley plants. Phytochem. 10: 109—116.

Harper D. B., Austin D. J., Smith H. 1970. The photocontrol of precursor incorporation into the *Pisum sativum* flavonoids. Phytochem. 9: 497—505.

Hänsel R., Hörhammer L. 1954. Vergleichende Untersuchungen über die Flavonglykoside der Betulaceen. Arch. Pharm. 287/59: 117—123.

Hörhammer L., Hänsel R., Frank P. 1953. Isolierung von Rutin aus *Betula humilis*. Arch. Pharm. 286/58: 33—34.

Maier V. P., Hasegawa S. 1970. L-Phenylalanine ammonia-lyase activity and naringenin glycoside accumulation in developing grapefruit. Phytochem. 9: 139—144.

McClure J. W. 1970. Secondary constituents of aquatic angiosperms. [In:] Harborne J. B. (ed.). Phytochemical Phylogeny. London, Acad. Press: 255—259.

Pawłowska L. msc.). Badania nad zróżnicowaniem związków flawonoidowych w liściach *Betula verrucosa, B. oycoviensis, B. „nova", B. pubescens, B. humilis i B. nana* (not published).

Smith H., Attridge T. H. 1970. Increased phenylalanine ammonia-lyase activity due to light treatment and its significance for the mode of action of phytochrome. Phytochem. 9: 487—495.

Smith H., Harper D. B. 1970. The effects of short- and long-terms irradiation on the flavonoid complement of the terminal buds of *Pisum sativum* var. Alaska. Phytochem. 9: 477—485.

Zucker M. 1969. Induction of phenylalanine ammonia-lyase in *Xanthium* leaf disks. Photosynthetic requirement and effect of daylength. Plant Physiol. 44: 912—922.

Zucker M. 1970. Rate of phenylalanine ammonia-lyase synthesis in darkness. Biochim. Biophys. Acta 208: 331—333.

*Author's address:*

*mgr Lucyna Pawłowska*
*Institute of Botany*
*Polish Academy of Sciences*
*Lubicz Str. 46; 31-512 Cracow; Poland*

## *Ilościowe zmiany dzienne glikozydów flawonolowych w liściach Betula humilis Schrk.*

Streszczenie

Glikozydy flawonolowe (rutyna i hyperozyd) izolowano ze świeżych liści jednego okazu *Betula humilis*. Zbioru dokonywano co godzinę, a w okresach szczytów co 15 minut, w ciągu kilku dni, w miesiącach letnich (lipiec — wrzesień). W ciągu dnia zaobserwowano dwa szczyty wzrostu ilości badanych glikozydów flawonolowych: pierwszy w godzinach rannych 9.00—10.00, drugi równy lub nieco wyższy od pierwszego w godzinach 13.00—14.00. Przebieg zmian zawartości obu badanych związków był równoległy, a jego charakter niezależny od daty zbioru materiału.