Elytra, Tokyo, **24** (2): 293–297, November 15, 1996

Analysis of Wood Sugars in the Dead Wood Eaten by the Larvae of *Ceruchus lignarius* and *Prismognathus angularis* (Coleoptera, Lucanidae) by Gas-liquid Chromatography

Kunio ARAYA

Graduate School of Human and Environmental Studies, Kyoto University,
Yoshida, Sakyo, Kyoto, 606–01 Japan

Abstract The sugar contents in the dead wood eaten by the larvae of *C. lignarius* and *P. angularis* are analyzed by using gas-liquid chromatography (GLC). The content of xylose in all the *C. lignarius* samples is markedly small, whereas those in the samples of *P. angularis* is relatively large. The contents of alkaline extractives and total wood sugars in all the samples of *C. lignarius* correspond to those of the advanced decay stage of brown rotten wood, and those of the samples of *P. angularis* to those of various stages of white rotten wood.

Both *Ceruchus lignarius* and *Prismognathus angularis* are small-sized lucanid beetles with overlapped distribution in the Cool-temperature Zone of Japan. The previous studies (ARAYA, 1993 a–b) revealed that *C. lignarius* is stenophagous for advanced decay stage of brown rot, whereas *P. angularis* is euryphagous for both decay stages and decay types.

Holocellulose is the total sugar content in wood cell wall and is a mixture of both cellulose and hemicellulose. Of these, cellulose consists solely of glucose, whereas hemicellulose is non-cellulosic polysaccharides comprising various sugars such as glucose, galactose, mannose and xylose. Compared with cellulose, hemicellulose is easily decomposed by wood decaying fungi, and composition of each hemicellulose fraction changes in a characteristic fashion depending on both the decay type and stage (TAKAHASHI, 1986). Thus, detailed analysis of wood sugars is needed to know precisely relationship between the occurrence of lucanid larvae and chemical properties of the decayed wood. In the present study, the sugar contents in the dead wood eaten by the larvae of *C. lignarius* and *P. angularis* are analyzed by using gas-liquid chromatography (GLC).

Materials and Methods

Sample materials. Four pieces of wood in which the larvae of *C. lignarius* occurred and four in which *P. angularis* occurred were collected randomly at Inabu-chô, northeastern Aichi Prefecture, the same place as in the previous studies in 1990

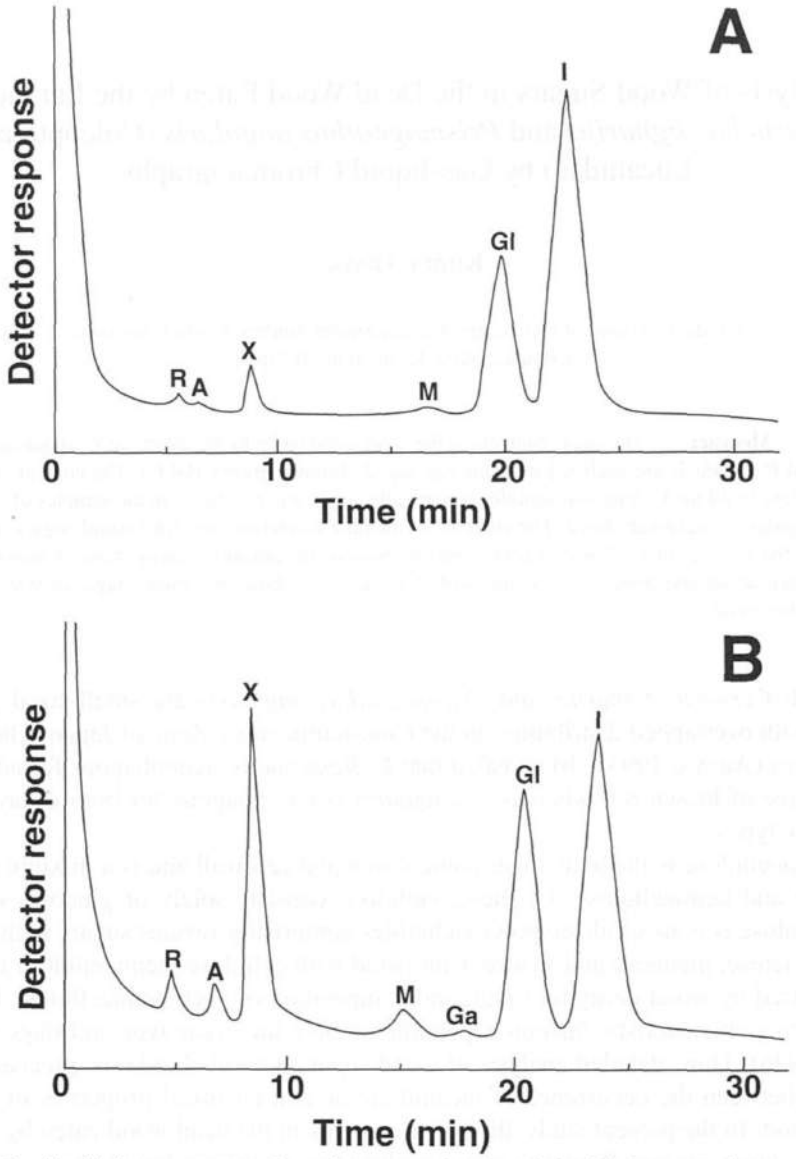

Fig. 1. Gas-liquid chromatograms of wood sugars analyzed as their alditol acetates in a sample of *C. lignarius* (top, sample No. 1) and a *P. angularis* (bottom, No. 5) with their relative retention times to inositol (t_R). R=rhamnose, t_R=0.15; A=arabinose, t_R=0.23; X=xylose, t_R=0.30; M=mannose, t_R=0.65; Ga=galactose, t_R=0.74; Gl=glucose, t_R=0.81; I=inositol (internal standard).

(ARAYA, 1993 a–b). Decay types and wood species were identified by macroscopic observation for each sample. A small wood piece was taken from the vicinity of a 3rd (final) instar larva in the collected dead wood piece. The wood piece was ground into

Table 1. The results of analysis of chemical components in the wood samples.

Samples code	Lucanid species	Tree species	Decay type	Rhamnose (%)	Arabinose (%)	Xylose[a] (%)	Mannose (%)	Galactose (%)	Glucose (%)	Total sugar (%)	Alkaline[a] extractives (%)
1	Cl	Fc	B.R.	0.3	N.D.	1.6	0.2	N.D.	14.3	16.4	77.4
2	Cl	Fc	B.R.	N.D.	N.D.	2.2	N.D.	0.1	18.3	20.6	77.2
3	Cl	Qm	B.R.	N.D.	N.D.	1.8	1.3	0.2	16.2	19.5	79.8
4	Cl	Bp	B.R.	0.5	0.3	2.7	0.6	N.D.	12.0	16.1	77.5
5	Pa	Fc	W.R.	1.0	1.0	15.7	1.2	N.D.	26.9	45.8	33.4
6	Pa	Qm	W.R.	0.6	N.D.	3.3	N.D.	N.D.	14.0	17.9	50.7
7	Pa	Qm	W.R.	0.6	0.6	13.3	1.0	N.D.	16.9	32.4	45.1
8	Pa	Bp	W.R.	1.5	1.3	22.0	N.D.	N.D.	26.9	51.7	33.9
9	—	Fc	S.W.	0.8	0.35	16.1	0.8	N.D.	39.0	57.0	22.1

Cl=*Ceruchus lignarius*; Pa=*Prismognathus angularis*; Fc=*Fagus crenata*; Qm=*Quercus mongolica*; Bp=*Betula platyphylla*; B.R=brown rot.; W.R.=white rot.; S.W=sound wood.; N.D=not determined.
[a]significantly different between the samples of *C. lignarius* and *P. angularis* ($p<0.05$, MANN-WHITNEY's U-test).

meal, and then contents of wood sugars and alkaline extractives were analyzed for each sample. The analysis was also made for a piece of sound wood of the Japanese beech, *Fagus crenata*.

Analysis of alkaline extractives. Alkaline extractives were calculated from weight loss after refluxing the wood meal with 1% sodium hydroxide solutions for one hour at 100°C. These include extraneous components (protein, pectin, tannin, etc.), low-molecular weight lignin and hemicellulose.

Analysis of wood sugars. Lipophilic components were removed from the wood meal by extraction with ethanol/benzene (1/1) in the same way as in the previous study (ARAYA, 1993 b), and then the wood meal was hydrolyzed with 72% sulfuric acid. Acid hydrolysates, which include wood polysaccharides, were reduced with sodium borohydride, and then the reduced monosaccharides, i.e. alditols, were analyzed by GLC after acetylation: alditol-acetate method (see BORCHARD & PIPER, 1970; SHINODA & INABA, 1985 for detail). Inositol solution was added as the internal standard (Alditol-Acetates Method, see SHINODA & INABA, 1985 for detail). GLC analysis was performed on a Shimadzu GC–14 Gas Chromatograph equipped with FID detector. The alditol acetates were separated on a glass column (2 m×3.0 mm I.D.) packed with 3% ECNSS–M coated on Gas Chrom Q (60–80 mesh). Carrier gas: N_2, 1.25 kg/cm^2, column temp.: 190°C, injection and detection temp.: 230°C. The contents of each sugar are calculated by using the formula shown by BORCHARD and PIPER (1970).

Estimation of decay stage. Decay stage was estimated by comparing the contents of alkaline extractives and the total wood sugars (the latter was nearly equivalent to the holocellulose contents) of each sample with those of the typical both brown and white rotten woods (TAKAHASHI, 1986; ARAYA, 1993 b).

Results and Discussion

Representative gas-liquid chromatograms for each species are shown in Fig. 1, and the results of the analyses are listed in Table 1. All the results are presented as the percentage to the moisture free samples weight.

All the materials from the wood eaten by *C. lignarius* contained significantly larger amounts of alkaline extractives (77.2 to 79.8%) than those eaten by *P. angularis* (33.4 to 50.7%) (MANN-WHITNEY's U-test, $p<0.05$).

In both *C. lignarius* and *P. angularis* samples, the contents of galactose, mannose, arabinose and rhamnose were quite small and not determinable in some samples. This is because the contents of these sugars were also quite small in the sound wood. The glucose contents of *P. angularis* samples (14.0 to 26.9%) were relatively larger than those of *C. lignarius* (12.0 to 18.3%), but no significant difference was detected. The content of xylose in all the *C. lignarius* samples was markedly small (1.6 to 2.7%), whereas those in the samples of *P. angularis* was relatively large (3.3 to 22.0%) though it varied considerably. Significant difference in the xylose contents was detected between the samples of two lucanid species (MANN-WHITNEY's U-test, $p<0.05$).

The contents of alkaline extractives and total wood sugars in all the samples of *C. lignarius* corresponded to those of the advanced decay stage of brown rotten wood (total weight loss 40 to 50%), whereas those of the samples of *P. angularis* to those of various stages of white rotten wood (total weight loss 20 to 60%). These results agree well with those of the previous study (ARAYA, 1993 b).

Brown rotten wood is fewer in frequency than white rotten one in cool temperature forest (ARAYA, 1993 a). It is known that brown rotten wood of the middle and advanced decay stages contains much greater amount of lignin than white rotten wood does (TAKAHASHI, 1986; ARAYA, 1993 b). Lignin is generally regarded as a quantitative digestion inhibiting factor for insects (OGUSHI, 1992). Further, as shown in the present study, brown rotten wood contained markedly small amounts of wood sugars which are regarded as calorific nourishments. These facts suggests that brown rotten wood (at least that eaten by *C. lignarius*) is inferior to white rotten wood as diet. If so, it is rather strange that *C. lignarius* is stenophagous for brown rotten wood in spite of its inferiority as diet.

Anyway, further rearing experiments of larvae using the brown and white rotten wood of various decay stages will be necessary to confirm the above-mentioned suggestion.

Acknowledgments

I wish to thank Prof. K. MURAKAMI and Dr. F. NAKATSUBO, Kyoto University, for technological support, and Dr. M. KON, Shiga Prefectural University, for his critical reading of the manuscript. I also thank Emeritus Prof. T. HIDAKA, Kyoto University, for his kind advice and encouragement during the course of this study. This study is sup-

ported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan, and JSPS Research Fellowships for Young Scientists.

要　約

荒谷邦雄：ガスクロマトグラフィーによるツヤハダクワガタとオニクワガタの発生している腐朽材中の木材構成糖の分析．—— ツヤハダクワガタとオニクワガタの発生している腐朽材の成分化学的な特性を調べるために，材中の木材構成糖をガスクロマトグラフィーによって分析・定量した．また，同時にそれぞれの材について，腐朽のタイプとその進度を推定するのに有効なアルカリ抽出物量の測定も行った．

ツヤハダクワガタの発生している腐朽材中の木材構成糖類は，健全材と比べるといずれもかなり減少していたが，とくにキシロースの減少がいちじるしかった．これに対して，オニクワガタの発生している腐朽材では，材によって差はあったが，ツヤハダクワガタの場合に比べると，糖類全体の減少の程度は低く，どの糖類も一様に減少していた．アルカリ抽出物量は，ツヤハダクワガタの材でいちじるしく多く，オニクワガタの材では，材によってばらつきがあったものの比較的少なかった．これらの分析結果は，ツヤハダクワガタが褐色腐朽の末期の状態にある材に特異的に穿孔すること，また，オニクワガタは主として白色腐朽のかなり幅広い腐朽進度にある材に穿孔することをあらためて裏づけるものである．

ところで，褐色腐朽材は，白色腐朽材に比べると出現頻度が低いだけでなく，昆虫にとって量的阻害物質となるリグニンを多量に含んでいる．そのうえ，今回の結果から，ツヤハダクワガタが特異的に穿孔する褐色腐朽材は，代謝のエネルギー源として消化・利用され得る木材構成糖類の含有量が低く，白色腐朽材と比べるに栄養面でもかなり劣ったものであることも示唆された．ツヤハダクワガタが，なぜこのように餌資源として質の良くない褐色腐朽材を選好するのか非常に興味深い．もしかすると，褐色腐朽材におけるキシロースの含有量の低さがなにか関係しているのかもしれない．いずれにせよ今後は，さまざまな腐朽進度にある褐色・白色腐朽材が幼虫の生存や成長に与える影響について，飼育実験で確認することが不可欠であろう．

References

ARAYA, K., 1993 a. Relationship between the decay types of dead wood and occurrence of lucanid beetles (Coleoptera: Lucanidae). *Appl. Ent. Zool.*, **28**: 27–33.

——— 1993 b. Chemical analyses of the dead wood eaten by the larvae of *Ceruchus lignarius* and *Prismognathus angularis* (Coleoptera: Lucanidae). *Ibid.*, **28**: 353–358.

OGUSHI, T., 1992. Resource limitation on insect herbivore populations. *In* HUNTER M. D., T. OGUSHI & P. W. PRICE (eds.), *Effects of Resource Distribution on Animal-Plant Interactions*, pp. 199–241. Academic Press, New York.

SHINODA, Y., & M. INABA, 1985. Chemistry of wood components. *In*: *An Experimental Text Book for Wood Science*: *2 Chemistry*, pp. 181–190. Chugaisangyo-Chosakai, Tokyo. (In Japanese.)

TAKAHASHI, M., 1986. Fungal decay types, their significance in wood preservation. *Wood Research and Technical Notes*, **22**: 19–36. (In Japanese.)