J Wood Sci (2001) 47:406–409

NOTE

Abul Kalam Azad · Koichi Ogiyama · Takeshi Sassa

Isolation of (+)-catechin and a new polyphenolic compound in Bengal catechu

Received: January 27, 2000 / Accepted: October 6, 2000

Abstract Methanol extractives from the red heartwood of Bengal catechu (*Acacia catechu*) contained (+)-catechin as a major component making up 0.3% of the wood. A new polyphenolic compound with a (+)-homo-iso-catechin structure and having catechol and phloroglucinol moieties constituted 0.005% of the wood, and probably its epimeric compound in trace amounts, were also found.

Key words *Acacia* spp. · Bengal catechu · (+)-Catechin · (+)-Homoisocatechin · Khair

Introduction

Acacia catechu is the most available tree species in southern Asian countries, especially Bangladesh, India, and Myanmer. Bengal catechu derives from *Acacia catechu*, which locally is called a khair tree and has red heartwood. The major constituent of the aqueous extractives is called catechu, whose local name is gilla khair or lila khair, but it has not been investigated phytochemically.[1,2] In this paper we report on the isolation and structural elucidation of a new polyphenolic compound together with a well-known (+)-catechin derived from it.

A.K. Azad · K. Ogiyama (✉) · T. Sassa
Faculty of Agriculture, Yamagata University, Tsuruoka, Yamagata 997-8555, Japan
Tel. +81-235-28-2922; Fax +81-235-28-2922
e-mail: kogiyama@tds1.tr.yamagata-u.ac.jp

This report was presented at the 49th Annual Meeting of the Japan Wood Research Society, Tokyo, April 1999 and the 44th Lignin Symposium, Gifu, October 1999

Experimental

Plant material

Heartwood of Bengal catechu was harvested in February 1997 at Rajshai, Bangladesh and chipped there. After being air-dried for 2 weeks, the chips were transported to Japan.

Isolation of the compounds

The heartwood (790g) was extracted with methanol (MeOH), three times for a 3-week interval at room temperature. The solution was evaporated to dryness under reduced pressure to give a brown solid (68.22g). A portion of the extractives (15g) was partitioned between ethyl acetate (EtOAc) and H_2O seven times. The EtOAc layer was separated, dried over anhydrous Na_2SO_4, and concentrated in vacuo to afford a deep red-brown residue (12.24g). The residue was separated into seven fractions on a Sephadex LH-20 column (Pharmacia) and eluted with 99% ethanol (EtOH). After the fifth fraction was evaporated to dryness, the residual solid (4.73g) was applied to a polyamide column (C-200, 75–150mesh; Wako Pure Chemical Indusries) and eluted with 2-propanol/H_2O (5:1, v/v), yielding a light brown crystalline residue (1.03g). Recrystallization from hot H_2O afforded compound 1 as needles (288mg). The mother liquor of the recrystallization process was further separated on a polyamide column using benzene/EtOAc (7:5, v/v) as eluent, and the fractions containing compound 2 were collected. These fractions were further purified with preparative thin-layer chromatography (TLC) using benzene/EtOAc/acetic acid (AcOH) (7:5:2, v/v/v) (method [A]) as a developing solvent, affording compound 2 as an amorphous solid.

General analytical methods of the compounds

Thin-layer chromatographic analyses were performed on precoated Kieselgel 60 F_{254} plates (E. Merck) using the

developing solvent benzene/EtOAc/AcOH (7:5:2, v/v/v) [A]. The compounds were detected under ultraviolet (UV) light irradiation or by spraying with diazotized sulfanilic acid or by spraying with 50% H_2SO_4 followed by heating at 120°C for 10 min. The ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were measured with a JEOL JNX-400 spectrometer at 400 and 100 MHz, respectively. Chemical shifts are given as δ (ppm) values with reference to CH_3OH and residual water in CD_3OD as the internal standard. After the compounds were trimethylsilylated with TMSI-H and *N,O*-bis(trimethylsilyl)-acetamide (GL Sciences), their GC-MS spectra were obtained with a Shimadzu GC-17A gas chromatograph directly coupled with a QP-5000 mass spectrometer [column: DB-1 capillary (30 m × 0.32 mm) (J&W Scientific); oven temperature: programmed as 50°C for 1.5 min, 50° to 240°C (rate of temperature rise 4°C/min); injection temperature 250°C; carrier gas: He. Electron ionization mass spectrometry (EI-MS) values were determined at 70 eV. The fourier transform infrared (FT-IR) spectra were obtained in a KBr disk with a Horiba FT-710 spectrophotometer. The UV spectral data were recorded with a Shimadzu UV-1600 PC spectrophotometer in MeOH solution.

Properties of compound 1

Compound 1 comprised fine needles recrystallized from H_2O, mp. 174°–176°C. It had an Rf of 0.45 by TLC according to method [A]. The optical rotation was $[\alpha]_{25.7}^{D}$ + 17 [50% acetone; c = 2.8 mg/ml]. UV, IR, and ^1H-NMR, and ^{13}C-NMR of compound 1 were identical with those of authentic (+)-catechin. GC-MS analysis of its trimethylsilane (TMS) ether were identical to the corresponding derivatives of (+)catechin. Methyl and acetyl derivatives of compound 1 had the same spectrographic properties as the corresponding derivatives of (+)-catechin. Thus, compound 1 was identified with (+)-catechin.

Properties of compound 2

Compound 2 (Fig. 1) was obtained as a chromatographically pure amorphous powder with an Rf of 0.69 by TLC according to method [A]. Optical rotation was $[\alpha]_{31.3}^{D}$ + 7.9 [MeOH; c = 1.4 mg/ml]. UV λ_{max}^{MeOH}nm (ε) 289 (10250). FT-IR $\nu_{max}^{KBr}cm^{-1}$ (%): 3433, 2922, 2852, 2295, 1637, 1552. ^1H-NMR, ^{13}C-NMR, and the related spectra of compound 2 are shown in Tables 1 and 2. GC-MS analysis of its TMS ether indicated a main peak at Rt = 18.29 min (compound 2–TMS) and another peak at 17.23 min (compound 3–TMS) (Fig. 2). EI-MS of compound 2-TMS m/z (relative intensity): 664 [M^+](36), 649(53), 635(12), 592(7), 577(7), 561(6), 368(100), 297(11), 281(10), 267(31), 249(17), 207(6), 191(23), 179(34), 147(30), 133(15).

EI-MS of compound 3–TMS

Compound 3 was found during GC-MS analysis of a TMS derivative of compound 2 in trace amounts: m/z (relative intensity): 664[M^+](7), 649(7), 368(73), 297(2), 281(2), 267(5), 249(3), 207(3), 179(6), 133(3), 73(100).

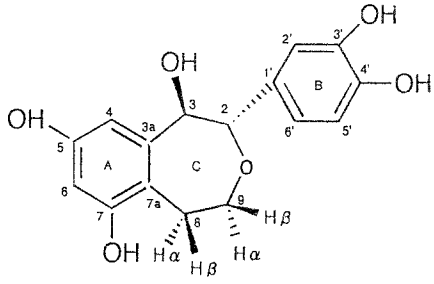

Fig. 1. Structure of compound 2

Table 1. ^1H NMR, HMQC, COSY, and HMBC spectral data for compound 2

Position	^1H-NMR	HMQC (^1H-^{13}C)	COSY (^1H-^1H)	HMBC C-position
1H-2	4.90 (d, $J_{2,3}$ = 11.7 Hz)	85.92	H-3	C-2
1H-3	4.49 (d, $J_{3,2}$ = 11.7 Hz)	74.47	H-2	C-3, C-2, C-1′
1H-8α	1.40–1.52 m	30.91	H-8β, H-9β	C-9
1H-8β	1.54–1.62 m	30.91	H-8α, H-9α	–
1H-9α	2.14–2.19 m	40.97	H-8β	C-8
1H-9β	3.12–3.23 m	40.97	H-8α	C-8
1H-4	5.91 (d, $J_{4,6}$ = 1.96 Hz)	97.08	H-6	C-4, C-5, C-3a, C-7a
1H-6	5.87 (d, $J_{6,4}$ = 1.96 Hz)	98.12	H-4	C-4, C-7, C-7a
1H-2′	6.95 (d, $J_{2',6'}$ = 1.96 Hz)	116.70	H-6′	C-2′, C-3′, C-4′, C-6′
1H-5′	6.79 (d, $J_{5',6'}$ = 8.30 Hz)	116.90	H-6′	C-5′, C-4′, C-3′, C-1′
1H-6′	6.84 (dd, $J_{6',5'}$ = 8.30 and $J_{6',2'}$ = 1.96 Hz)	121.69	H-5′, H-2′	C-6′, C-5′, C-1′, C-2′

Solution in CD_3OD referenced to: (1) CH_3OH at δH 3.30 (^1H), and (2) residual water at δH 4.86 (^1H) and δC 49.80 (^{13}C)

NMR, nuclear magnetic resonance; HMQC, heteronuclear multiple-quantum correlation; COSY, correlated spectroscopy; HMBC, heteronuclear multiple bond connectivity

Table 2. ^{13}C NMR spectral data for compound 2

Position	^{13}C NMR	DEPT
2	85.92	CH
3	74.47	CH
3a	169.50	C
4	97.08	CH
5	166.09	C
6	98.12	CH
7	165.31	C
7a	102.65	C
8	30.91	CH_2
9	40.97	CH_2
1′	130.67	C
2′	116.70	CH
3′	147.93	C
4′	147.11	C
5′	116.90	CH
6′	121.69	CH

Solution in CD_3OD referenced to: (1) CH_3OH at δH 3.30 (^{1}H) and (2) residual water at δH 4.86 (^{1}H) and δC 49.8 (^{13}C)
DEPT, distortionless enhancement by polarization transfer

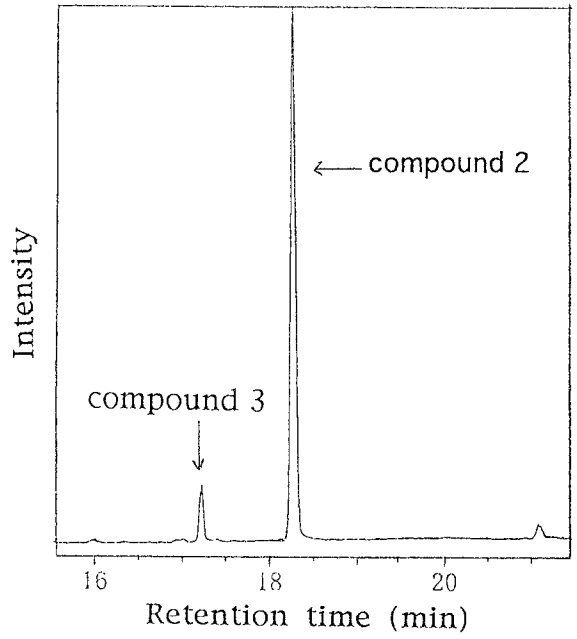

Fig. 2. Gas chromatographic analysis of trimethylsilane (TMS) derivative of compound 2, where a trace amount of compound 3 was obtained as an impurity

Results and discussion

General features of the major extractives

Chromatographic separation of the MeOH extractives of the red heartwood of Bengal catechu by Sephadex LH-20, polyamide columns, and silica gel preparative thin layers resulted in isolation of compound 1 and a new compound 2. As all of the spectrographic and physical properties of compound 1 were consistent with those of authentic (+)-catechin, it was identified as (+)-catechin.[3–5] Oligomeric proanthocyanidins, which are common in *Acacia* species, were not found in this experiment. An unidentified compound (compound 2) was isolated as a chromatographically pure compound from the mother liquor of the recrystallization of (+)-catechin. The compound was polyphenolic in nature, as shown by its UV absorption spectrum at λ_{max} 289 nm and red color after reaction with diazotized sulfanilic acid.

The ^{13}C-NMR spectrum of compound 2 indicated the presence of 12 aromatic and 4 aliphatic carbons in the molecule. Among them, four aromatic and one aliphatic carbons linked with hydroxyl groups, as described below. The ^{1}H-NMR spectrum also indicated the presence of five aromatic protons and six aliphatic protons. Because the trimethylsilane (TMS) ether derivatives of compound 2 indicated a major peak with [M^+] at m/z 664 during GC-MS (refer to Fig. 2), the rest element was assumed to be an etherial oxygen. Thus the molecular formula of compound 2 was assumed to be $C_{16}H_{16}O_6$ ($C_{16}H_{11}O(OH)_5$). Two aromatic protons that appeared as *meta*-coupled doublets in the A-ring [δ 5.87 (1H, d, $J_{6,4}$ = 1.96 Hz, assigned as H-6 in Fig. 1); δ 5.91 (1H, d, $J_{4,6}$ = 1.96 Hz, H-4 in Fig. 1)], and both were suggested to be methine carbons from distortionless enhancement by polarization transfer (DEPT), as shown in Tables 1 and 2. The ^{1}H-NMR spectrum also showed the presence of other three aromatic protons at δ 6.79 [1H, d, $J_{5',6'}$ = 8.30 Hz, assigned as H-5′ in Fig. 1], δ 6.84 (1H, dd, $J_{6',2'}$ = 1.96 and $J_{6',5'}$ = 8.30 Hz, H-6′] and δ 6.95 (1H, d, $J_{2',6'}$ = 1.96 Hz, H-2′ in Fig. 1]; they were also aromatic methine carbons according to DEPT. Because the splitting pattern of these aromatic protons resembled those of (+)-catechin, they were assigned as protons in rings A and B, as shown in Fig. 1.

The two doublets appeared at δ 4.90 (1H, d, $J_{2,3}$ = 11.71 Hz, H-2) and δ 4.49 (1H, d, $J_{3,2}$ = 11.71 Hz, H-3)]. They were suggested to be aliphatic methine carbons according to DEPT. The large J value suggests that the *vicinal* protons are in the *trans* configuration.[6,7] The two distinct bands at 2922 and 2852 cm^{-1} during FT-IR suggested that there are methylene groups in the compound. The presence of two methylene groups was also indicated by DEPT. The four multiplets at δ 1.40–1.52, 1.54–1.62, 2.14–2.19, and 3.12–3.23 each corresponded to one proton and were due to coupling with each of the other four protons. Their presence was also supported by ^{1}H-^{1}H COSY.

Assignment of the carbon signals was confirmed by DEPT and ^{13}C-^{1}H HMQC (Fig. 3) analyses (Tables 1,2). Significant long-range coupling observed in the HMBC and ^{1}H-^{1}H COSY spectra are shown in Table 1. Although limited amounts of compound 2 resulted in failure to elucidate its structure completely, the information we have suggests that the compound would have a novel seven-member heterocyclic ring C corresponding to a homo-iso-catechin structure, as shown in Fig. 1. Investigation of the TMS derivative of compound 2 by GC-MS revealed the existence of another compound in trace amounts, with the same molecular weight and similar mass fragmentation pattern as compound 2. It is still unclear whether it occurred naturally or artificially during TMS

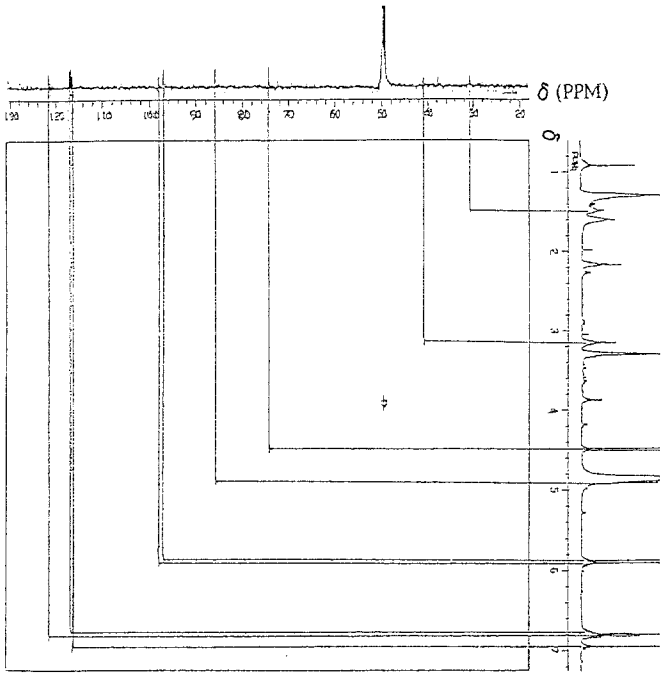

Fig. 3. ^{13}C-^{1}H correlated spectroscopy (COSY) [heteronuclear multiple-quantum correlation (HMQC)] spectrum of compound 2

derivatization, but it is suggested to be an epimer of compound 2.

Acknowledgment The authors are grateful to Dr. Md. Anwarul Islam, Horticulture Research Center, Bangladesh Agriculture Research Institute, Gazipur, Bangladesh for identifying and providing the plant material.

References

1. Ali MS, Zaman MB, Islam MN, Mondal IH (1982) Improvement in the conventional method of extraction of catechu (Khair). Bangladesh J Sci Ind Res 17:126–129
2. Ali MS, Hye MA, Mondal MIH, Rahman MA (1991) Extraction of catechu from different layers of the stem of *Acacia catechu* tree. Bangladesh J Sci Ind Res 26:171–174
3. Samejima M, Yoshimoto T (1979) Procyanidins from the inner bark of Sugi (*Cryptomeria japonica* D. Don). Mokuzai Gakkaishi 25:671–677
4. Foo LY, Karchesy JJ (1989) Procyanidin dimers and trimers from Douglas fir inner bark. Phytochemistry 28:1743–1747
5. Oshima Y (1963) Encyclopaedia chimica, 2nd edn. Kyoritsu Press, Tokyo, p 447
6. Haslam E (1988) Plant polyphenols: vegetable tannins revisited. In: Proanthocyanidins. Cambridge University Press, Cambridge, p 25
7. Williams DH, Fleming I (1980) Spectroscopic methods in organic chemistry, 2nd edn. McGraw-Hill, New York, p 100