J. Indian Chem. Soc.,
Vol. 79, June 2002, pp. 553-554

NOTE

Chemical constituents of *Diospyros nigra*

B. Dinda[a], S. K. Das[a], A. Bhattacharya[a], U. C. De[a] and Y. Harigaya[b]

[a]Department of Chemistry, Tripura University, Agartala-799 130, India

E-mail : dindabtu@rediffmail.com *Fax* : 91-0381-37-4801

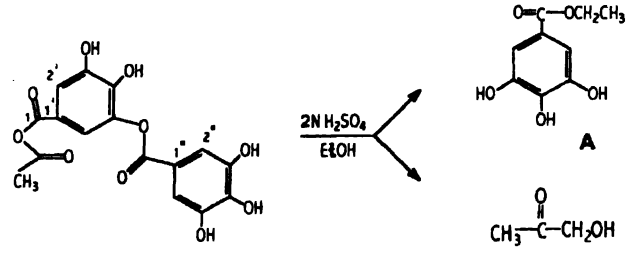
[b]School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo 108 8641, Japan

Manusaript received 11 May 2001, revised 13 September 2001, accepted 25 September 2001

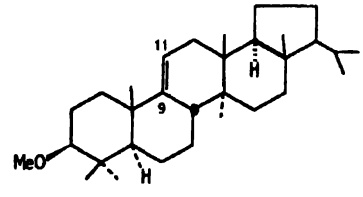
Two new compounds 1-*O*-bisgalloylhydroxyacetone (1) and cylindrin epoxide (3) along with five known compounds, viz. gallic acid, cylindrin (2), lupeol, betulin and betulinic acid have been isolated from different parts of *Diospyros nigra*.

Diospyros nigra Pierre (Syn. *D. sapota* Roxb.)[1] of family Ebenaceae, an American plant is cultivated at Agartala as an avenue tree. The bark of this plant is antirheumatic and also used in swellings. The fruit is antidysenteric and the leaf is laxative, styptic and antileucorrhoeic[2]. Phytochemical investigation on different parts of the plant has been carried out by us for the first time.

Air-dried pddwered stem-bark of *D. nigra* (1 kg), collected from M. B. B. College campus, Agartala, was extracted with EtOAc. The EtOAc extract was concentrated to a dark brown semi-solid mass (8.5 g) by distillation of solvent under reduced pressure. The semi-solid mass was subjected to column chromatography over silica gel (mesh 60–120). Elution of the column with petroleum ether (b.p. 60–80°) (PE)-C_6H_6 (9 : 1) gave a white solid which on crystallization from PE-C_6H_6 afforded white needles (10 mg), $C_{30}H_{50}O$ (M^+ 426), m.p. 212°, $[\alpha]_D$ + 25° ($CHCl_3$), which showed positive LB test. It was identified as lupeol[3] by direct comparison (co-TLC and m.m.p. undepressed) with an authentic sample. Elution with PE-C_6H_6 (1 : 4) afforded a solid which on crystallization from PE-C_6H_6 gave colourless needles (12 mg), $C_{30}H_{50}O_2$ (M^+ 442), m.p. 252°, characterized as betulin[3] by co-TLC, m.m.p. and comparison of spectral data with those of authentic sample. Elution of the column with C_6H_6-$CHCl_3$ (1 : 1) gave a white solid which was a mixture of three compounds as revealed by TLC. This mixture was subjected to column chromatography over silica gel. Elution of the column with C_6H_6-EtOAc (6 : 1) gave a soild which on crystallization from C_6H_6-EtOAc gave colourless needles of gallic acid (25 mg), m.p. 252°, identified by direct comparison (m.m.p. undepressed and co-TLC) with an authentic sample. Elution of the column with C_6H_6-EtOAc (2 : 1) gave colourless needles of DN-3 (40 mg), m.p. 235°. Elution of the column with C_6H_6-EtOAc (1 : 1) gave betulinic acid[3] as colourless needles by crystallization from MeOH, m.p. 315°, identified by comparison with an authentic sample (m.m.p. undepressed and co-TLC).



1



2

3 9,11-epoxy

DN-3, $C_{17}H_{14}O_{10}$ (M^+ 378), showed blue colouration with alcoholic $FeCl_3$, suggesting its phenolic nature. It exhibited λ_{max} (MeOH) at 218 (log ε, 4.41) and 275 (4.03) nm characteristic of gallates[4] and ν_{max} at 3460–3260 (OH), 1720 (C=O) and 1695 cm^{-1} (aromatic ester). Its ^1H NMR spectrum (acetone-d_6) displayed signals for methylene protons at δ 3.90 (2H, br s, H_2C-1), bisgalloyl protons at δ 7.18 (2H, s, H-2″,6″), 7.98 (1H, br s, H-2′) and 8.20 (1H, br s, H-6′), while in $CDCl_3$ containing a few drops of

methanol-d_4) showed an additional signal of ketomethyl ($COCH_2$) at δ 2.30 (3H, s). The ^{1}H NMR data corroborated[4] the bisgalloyl structure **1** for the compound. The upfield shift of oxymethylene protons was possibly due to shielding effects the aromatic ring and the ester carbonyl. The much downfield shift of one aromatic proton (H-6′) was possibly due to deshielding effect of both ester and ketone carbonyls. The GC-MS of the compound recorded some significant mass peaks at *m/z* 378 $[M]^+$ (13%), 335 $[M-43]^+$ (5), 321 (6), 305 (4), 225 (7), 170 (48), 153 (42), 126 (13), 57 (94) and 43 (100), also suggesting this structure. The compound on ethanolysis with 2*N* H_2SO_4 in dry ethanol under reflux for 6 h gave ethyl gallate (**A**), $C_9H_{10}O_5$ $[M]^+$ 198, m.p. 155°, and hydroxyacetone (**B**) $C_3H_6O_2$. The products ethyl gallate (**A**) was characterized by its spectral studies [δ (Me_2CO-d_6) 1.30 (3H, t, *J* 7 Hz, CH_3), 4.24 (2H, q, *J* 7 Hz, CH_2), 7.12 (2H, s, ArH); *m/z* 198 $[M]^+$ (15%), 170 (33), 153 (100), 125 (31)], and hydroxyacetone (**B**) by preparation of its DNP derivative, m.p. 129° and its comparison with the DNP derivative of an authentic sample in m.m.p. (undepressed) and co-TLC. Based on the foregoing evidence, compound DN-3 was characterized as 1-*O*-*bis*-*galloylhydroxyacetone* (**1**). To the best of our knowledge, it is a new natural product.

The EtOAc extract of air-dried leaves of *D. nigra* (2 kg) was column chromatographed over silica gel. Elution of the column with PE gave a solid which on crystallization from PE-C_6H_6 afforded needle shaped crystals (70 mg), m.p. 268°, $C_{31}H_{52}O$ $[M]^+$ 440, $[\alpha]_D$ + 58.8 (*c*, 1.16, $CHCl_3$), identified as cylindrin (**2**)[6] by comparison of its m.p. and spectral data (^{1}H NMR, ^{13}C NMR and EIMS) with those of authentic sample. It is the first report of cylindrin from genus *Diospyros*. Elution of the column with PE-C_6H_6 (5 : 1) gave a solid which on repeated chromatography afforded colourless crystals (12 mg), m.p. 248°, named DN-8. DN-8. $C_{31}H_{52}O_2$ (M^+ 456), showed positive LB test for triterpenoids. Its IR spectrum (KBr) showed the bands for *gem*-dimethyl (1380 and 1360 cm^{-1}) and epoxy function (1270, 1210 and 860 cm^{-1}). The 400 MHz ^{1}H NMR spectrum ($CDCl_3$) displayed signals for eight methyls at δ 0.72 (3H, s), 0.76 (6H, s), 0.82 (3H, d, *J* 6 Hz), 0.88 (3H, d, *J*, 6 Hz), 0.96 (3H, s), 1.16 (3H, s) and 1.24 (3H, s); three methine protons – one at δ 2.02 (1H, m, $-CH(CH_3)_2$), other at δ 2.65 (1H, dd, *J* 4, 12 Hz, H-3) and rest at δ 3.11 (1H, δ, *J* 6 Hz, H-11) and one methoxy at δ 3.33 (3H, s MeO-3), suggesting the compound to be epoxy derivative of cylindrin (**3**). The spectrum was similar to that of cylindrin except the disappearance of the signal of olefinic proton and appearance of methine proton signal at δ 3.11. The EI MS of the compound also corroborated this structure showing peaks at *m/z* 456 $[M]^+$ (23%), 441 $[M-Me]^+$ (6), 413 $[M-43]^+$ (6), 409 (441-MeOH, 6), 393 (4), 287 (3), 273 (4), 241 (3), 203 (14), 174 (15), 163 (10), 137 (19) and 43 (100). On the basis of the available informations, the compound was assigned the epoxycylindrin structure (**3**). However, further study is required to confirm the stereochemistry of epoxy function.

The concentrated EtOAc extract from air-dried fruits of *D. nigra* (0.5 kg) was subjected to column chromatography. Elution of the column with PE-C_6H_6 (6 : 1) gave lupeol as colourless needles (15 mg), m.p. 212°, while elution with C_6H_6-$CHCl_3$ (1 : 1) gave betulinic acid as colourless needles (30 mg), m.p. 312°.

Acknowledgement

The authors are thankful to I.I.C.B., Kolkata, for providing some spectral data. One of the authors (U.C.D.) is grateful to C.S.I.R., New Delhi, for a Senior Research Fellowship.

References

1. D. B. Deb, "The Flora of Tripura State", Today and Tomorrow's Publishers, New Delhi, 1981, Vol. 1, p. 426.
2. Personal communication from local tribal *Ojha* (physician).
3. B. Dinda, A. K. Hazra, S. K. Das, G. Chel, R. Chakraborty and B. C. Ranu, *Indian J. Chem., Sect. B*, 1995, **34**, 624.
4. S. A. M. Hussein, H. H. Barakat, I. Merfort and M. A. M. Nawwar, *Phytochemistry*, 1997, **45**, 819.
5. E. A. Haddock, R. K. Gupta, S. M. K. Al-Shafi and E. Haslam, *J. Chem. Soc., Perkin Trans. 1*, 1982, 2515.
6. J. W. Blint and M. H. G. Munto, *Org. Magn. Reson.*, 1980, **13**, 26; S. H. Goh, F. L. Lai and P. T. Gee, *Phytochemistry*, 1988, **27**, 877.