CHEMICAL EXAMINATION OF *PROSOPIS SPICIGERA* SEEDS

D. K. Bhardwaj, A. K. Gupta, R. K. Jain and G. C. Sharma

Department of Chemistry, University of Delhi, Delhi-110007 (India)

Abstract.—Examination of chemical constituents in the ethanolic extract of the seeds of *Prosopis spicigera* has led to the isolation of five known polyphenolics, gallic acid, patuletin, luteolin, patulitrin, and rutin, and a new flavone herein named prosogerin-E. Based on chemical and spectral data the constitution of prosogerin-E has been determined to be 6,7-dihydroxy-3',4',5'-trimethoxyflavone (**1**). Comparison of prosogerin-E ethyl ether with synthetic 6,7-diethoxy-3',4',5'-trimethoxyflavone (**1a**) showed them to be identical, thereby confirming the structure.

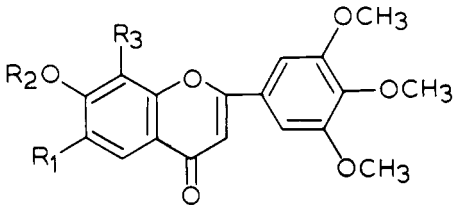
Prosopis spicigera Linn. (Leguminosae), a thorny tree known for medicinal properties (1), has been examined for its chemical constituents (2–6). This paper reports the isolation of six compounds from the ethanol extract of its seeds. Among these compounds, four are non-glycosidic polyphenolics, gallic acid, patuletin, luteolin, and a new compound named prosogerin-E. The other two compounds are glycosidic polyphenolics, patulitrin and rutin. Prosogerin-E, has been assigned the structure 6,7-dihydroxy-3',4',5'-trimethoxyflavone (**1**).

Prosogerin-E (**1**): Color reactions and spectral data showed prosogerin-E to be a flavone without any oxygen function at the C–3 position. Alkali fission produced tri-*O*-methylgallic acid, thereby fixing three methoxyls at C–3', C–4' and C–5' in the side phenyl of the molecule. Compound **1** yielded a diethyl ether (**1a**) and a diacetate (**1b**) indicating it to be a dihydroxy-3',4',5'-trimethoxyflavone. Quastel test (7) showed that these hydroxyls constituted an ortho-dihydroxy system which was substantiated by uv spectral shifts observed in the presence of sodium acetate-boric acid. In addition, its solubility in aqueous sodium carbonate (10%) indicated a free hydroxyl at C–7. Since prosogerin-E contained an ortho-dihydroxy system, two possible isomeric structures could be considered, viz., 6,7-dihydroxy-3',4',5'-trimethoxyflavone (**1**) and 7,8-dihydroxy-3',4',5'-trimethoxyflavone (**2**). The pmr spectrum of prosogerin-E acetate indicated five substituents, three methoxyls (δ 3.90, 9H) and two acetoxyls (δ 2.30, 6H), and five aromatic protons having signals at δ 6.70 (1H), 7.10 (2H), 7.55 (1H) and 8.00 (1H). The signal at δ 6.7 (1H) was characteristic (8) of the proton at C–3 in flavones, whereas the signal at δ 7.1 (2H) seemed to correspond to the two protons at C–2' and C–6'. Furthermore, the down-field singlet at δ 8.0 (1H) was attributed to the aromatic proton at C–5, indicating that C–6 was not unsubstituted. Another singlet at δ 7.55 (1H) was due to the aromatic proton at C–8. The signals corresponding to C–5 and C–8 protons appeared to be shifted down-field, probably due to the de-shielding effects of acetoxyls at C–6 and C–7, respectively. A priori, prosogerin-E could be considered more probably as 6,7-dihydroxy-3',4',5'-trimethoxyflavone (**1**) rather than 7,8-dihydroxy-3',4',5'-trimethoxyflavone (**2**). Hence prosogerin-E diethyl ether would be 6,7-diethoxy-3',4',5'-trimethoxyflavone (**1a**) and not 7,8-diethoxy-3',4',5'-trimethoxyflavone (**2a**). However, prosogerin-E showed hypsochromic shifts instead of the usual bathochromic uv spectral shifts for a C–7 hydroxyl when recorded in the presence of sodium acetate (8). This controversy was settled by comparison of the prosogerin-E diethyl ether with **1a** and **2a** obtained synthetically as described. This provided unequivocal confirmation of the structure (**1**) proposed for prosogerin-E.

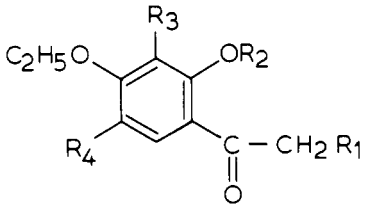
6,7-Diethoxy-3',4',5'-trimethoxyflavone (**1a**) required for comparison was synthesized with 2-(3',4',5'-trimethoxy)benzoyloxy-4,5-diethoxyacetophenone (**4**) as the essential intermediate. It was obtained by the condensation of 2-hydroxy-4,5-diethoxyacetophenone (**3**) (9) with tri-*O*-methylgalloyl chloride (10). When compound **4** underwent Baker-Venkataraman rearrangement (11–14), it gave the

β-diketone (**5**) which was cyclized by glacial acetic acid and fused sodium acetate to 6,7-diethoxy-3',4',5'-trimethoxyflavone (**1a**), identical with prosogerin-E diethyl ether.

Similarly 7,8-diethoxy-3',4',5'-trimethoxyflavone (**2a**) was synthesized with 2-(3',4',5'-trimethoxy)benzoyloxy-3,4-diethoxyacetophenone (**7**) obtained from 2-hydroxy-3,4-diethoxyacetophenone (**6**) (9) and tri-*O*-methylgalloyl chloride (10). Compound **7** was converted into the corresponding 7,8-diethoxy-3',4',5'-trimethoxyflavone (**2a**) through the β-diketone (**8**).



1 $R_1 = OH$; $R_2 = R_3 = H$
1a $R_1 = OC_2H_5$; $R_2 = C_2H_5$; $R_3 = H$
1b $R_1 = OAc$; $R_2 = Ac$; $R_3 = H$
2 $R_1 = R_2 = H$; $R_3 = OH$
2a $R_1 = H$; $R_2 = C_2H_5$; $R_3 = OC_2H_5$



3 $R_1 = R_2 = R_3 = H$; $R_4 = OC_2H_5$
4 $R_1 = R_3 = H$; R_2 = Tri-o-methylgalloyl; $R_4 = OC_2H_5$
5 R_1 = Tri-o-methylgalloyl; $R_2 = R_3 = H$; $R_4 = OC_2H_5$
6 $R_1 = R_2 = R_4 = H$; $R_3 = OC_2H_5$
7 $R_1 = R_4 = H$; R_2 = Tri-o-methylgalloyl; $R_3 = OC_2H_5$
8 R_1 = Tri-o-methylgalloyl; $R_2 = R_4 = H$; $R_3 = OC_2H_5$

EXPERIMENTAL[1]

PLANT MATERIAL.—The plant material was collected from the south Delhi area. It was identified by Dr. G. S. Paliwal, Reader in Botany, Department of Botany, University of Delhi, Delhi-7, as *Prosopis spicigera* Linn.

EXTRACTION AND PURIFICATION.—Air-dried seeds of *Prosopis spicigera* (10 kg) were exhaustively extracted with petroleum ether (60–80°), benzene and then ethanol. Solvent-free ethanolic extract was extracted first with ether and then with ethyl acetate. Since tlc examination of the ether and ethyl acetate soluble fractions were found to contain the same non-glycosidic components (gallic acid, patuletin, luteolin and prosogerin-E), these were combined and marked as fraction-x_1. After the removal of fraction-x_1, the residue containing two glyco-

[1]Melting points are uncorrected. Ir spectra were recorded on a Perkin-Elmer infracord-137 (ν max in cm^{-1}), uv spectra (λ max in nm) on a Beckman DU-2 spectrophotometer and pmr spectra on a Varian A-60 spectrometer with TMS as internal standard. Chemical shifts are given in δ (ppm), and $CDCl_3$ was used as solvent for recording pmr spectra. Diethyl sulfate or dimethyl sulfate in dry acetone with anhydrous potassium carbonate were used for alkylations, whereas acetic anhydride and dry pyridine were used for acetylations. Silica-gel was used as adsorbent in chromatographic examinations.

sidic components, patulitrin and rutin, was marked as fraction-x_2. Fractions x_1 and x_2 were separately subjected to column chromatography followed by preparative tlc and fractional crystallization to effect the separation and isolation of their chemical constituents.

The examination of fraction X_1 with silica gel as adsorbent and benzene-methanol-acetic acid (45:5:1) as solvent showed the presence of four major compounds, gallic acid, patuletin, luteolin and prosogerin-E. Fraction X_1 was subjected to column chromatography with silica gel as adsorbent and a number of eluents in order of increasing polarity. Elutions with benzene-ethyl acetate (60:40; 50:50; 40:60; 30:70; 20:80 and 10:90) gave a mixture of gallic acid and patuletin which were separated by preparative tlc with benzene-ethyl acetate (60:40) as the solvent system. Further elutions of the column with ethyl acetate-methanol (98:2; 97:3 and 95:5) gave a mixture of luteolin and prosogerin-E which were also separated by preparative tlc with benzene-methanol-acetic acid (45:5:1) as solvent.

Fraction-X_2 Tlc examination of fraction-X_2 with chloroform-methanol-water (35.5:13.5:1.8) showed the presence of patulitrin and rutin, which were separated by column chromatography as was done in case of fraction-X_1. Elutions with ethyl acetate-methanol (95:5; 90:10; 85:15 and 80:20) gave patulitrin, whereas subsequent elutions with ethyl acetate-methanol (70:30; 60:40 and 50:50) gave rutin.

GALLIC ACID.—Gallic acid crystallized from ethyl acetate-petroleum ether as colorless shining needles (170 mg), mp 253–54°. Color reactions, spectral data, and its derivatives showed it to be gallic acid.

PATULETIN.—Patuletin crystallized from ethanol as yellow prisms (135 mg), mp 262–63°. It gave an olive-green coloration with alcoholic ferric chloride and also red coloration with ammonium molybdate in acetic acid for the *ortho*-dihydroxy system (7); uv, λ max (MeOH): 260 and 370 nm; +$AlCl_3$: 275 and 450 nm; +$AlCl_3$+HCl; 270 and 430 nm; +NaOAc: 270 and 395 nm; +NaOAc+H_3BO_3: 270, and 385 nm. On acetylation it gave a pentaacetate which crystallized as colorless prisms from ethyl acetate-petroleum ether, mp 170–71°. On methylation, it gave a pentamethyl ether which crystallized as colorless needles from ethanol, mp 142–44°.

LUTEOLIN.—Luteolin crystallized from methanol as yellow needles (110 mg), mp 330–31°. It gave a greenish-brown coloration with alcoholic ferric chloride and red coloration with ammonium molybdate in acetic acid for the *ortho*-dihydroxy system (7); uv, λ max (MeOH): 260 and 345 nm; +$AlCl_3$; 270 and 420 nm; +$AlCl_3$+HCl: 270 and 390 nm; +NaOAc: 270 and 385 nm; +NaOAc+H_3BO_3: 265 and 380 nm. It gave a tetraacetate, colorless needles from ethyl acetate-petroleum ether, mp 226–27°, as well as a tetramethyl ether, colorless needles from benzene-petroleum ether, mp 193–94°.

PROSOGERIN-E (**1**).—Prosogerin-E crystallized from ethyl acetate-petroleum ether as yellow needles (80 mg), mp 265–66°, (*Anal.* Found: C, 62.7; H, 5.00. Calc. for $C_{15}H_{16}O_7$: C, 62.79; H, 4.68%). It gave a green coloration with alcoholic ferric chloride and a positive Quastel test (7). It gave an ir spectra, ν max 3448, 1618, 1582, 1462, 1342, 1282, 1241, 1121, 995, 963, 859 and 833 cm^{-1}; uv, λ max (MeOH): 280, 315 and 325 nm; +NaOAc: 270, 290 (sh), and 340 nm; +NaOAc+H_3BO_3: 290, 320 and 330 nm.

ALKALI DEGRADATION OF PROSOGERIN-E.—Prosogerin-E (15 mg) was refluxed with absolute ethanolic potassium hydroxide (8 ml, 50%) for 15 hr. The solvent was removed and then was acidified with dilute hydrochloric acid and extracted with ether. The ether layer was washed with aqueous sodium bicarbonate (5%). The aqueous layer was acidified and then extracted with ether. The ether soluble fraction, when examined by tlc with benzene-methanol-acetic acid (20:5:1) as solvent, was found to contain a substance identical with an authentic sample of tri-*O*-methylgallic acid.

PROSOGERIN-E ETHYL ETHER (**1a**).—Prosogerin-E on ethylation gave an ethyl ether (**1a**), which crystallized as colorless needles from chloroform-petroleum ether, mp 208–9°; pmr: δ 1.55 (m, 6H, 2X $-OCH_2CH_3$), 3.98 (s, 9H, 3X $-OCH_3$), 4.21–4.32 (m, 4H, 2X $-OCH_2CH_3$), 6.77 (s, 1H, C-3-H), 7.03 (s, 1H, C-8-H), 7.15 (s, 2H, C-2'-H & C-6'-H) and 7.60 (s,1H, C-5-H). (*Anal.* Found: C, 66.30; H, 5.80. Calc. for $C_{22}H_{24}O_7$: C, 65.99; H, 6.04%).

PROSOGERIN-E ACETATE (**1b**).—Prosogerin-E on acetylation gave an acetate (**1b**), which crystallized as colorless needles from chloroform-petroleum ether, mp 217–18° (*Anal.* Found: C, 62.00; H, 5.10; Calc. for $C_{22}H_{20}O_8$: C, 61.68; H, 4.71%). It gave the following spectral data: pmr: 2.30 (s, 6H, 2X $-OCOCH_3$), 3.90 (s, 9H, 3X $-OCH_3$), 6.70 (s, 1H, C-3-H), 7.10 (s, 2H, C-2'-H & C-6'-H), 7.55 (s, 1H, C-8-H) and 8.00 (s, 1H, C-5-H); ms (*m/e*): 344, 343, 316, 315, 192, 177, 153, 152.

2-(3',4',5'-TRIMETHOXY)BENZOYLOXY-4,5-DIETHOXYACETOPHENONE (**4**).—2-Hydroxy-4,5-diethoxyacetophenone (**3**; 2 g) (9) and tri-*O*-methylgalloyl chloride (2.5 g) (10) in dry pyridine (15 ml) were heated at 100° for 45 min. and left at room temperature for 6 hr. The reaction product was then worked up as usual. The ester (**4**) thus obtained crystallized from ethyl acetate-petroleum ether as colorless micro-needles (2.7 g), mp 139–40°, (*Anal.* Found: C, 62.80; H, 6.30. Calc. for $C_{22}H_{26}O_8$: C, 63.15; H, 6.26%).

2-HYDROXY-4,5-DIETHOXY-3',4',5'-TRIMETHOXYDIBENZOYLMETHANE (**5**).—The above ester (**4**; 2.5 g) in dry pyridine (20 ml), when warmed at 40–50° with powdered potassium hydroxide (2 g) for 45 min. with thorough shaking, underwent rearrangement (11–14) to give the β-diketone (**5**). It was worked up as usual. The reaction product crystallized from ethyl acetate-petroleum ether to give the β-diketone (**5**) as bright yellow needles (1.5 g), mp 130–31°.

It gave a green ferric reaction. (*Anal.* Found: C, 62.90, H, 6.60. Calc. for $C_{22}H_{26}O_8$: C, 63.15; H, 6.26%).

6,7-DIETHOXY-3',4',5'-TRIMETHOXYFLAVONE (**1a**).—The above β-diketone (**5**; 1 g) in glacial acetic acid (20 ml) and fused sodium acetate (1 g) was heated under reflux for 3 hr., cooled and then treated with ice. The reaction product thus obtained crystallized from chloroform-petroleum ether to give the compound (**1a**) as colorless needles (0.8 g), mp 208–9°. On direct comparison it was found to be identical with the ethyl ether of prosogerin-E.

2-(3',4',5'-TRIMETHOXY)BENZOYLOXY-3,4-DIETHOXYACETOPHENONE (**7**).—2-Hydroxy-3,4-diethoxyacetophenone (**6**; 2 g) (9) in dry pyridine (20 ml) and tri-*O*-methylgalloyl chloride (2.5 g) (10) were heated at 100° for 45 min. to obtain the ester (**7**) which crystallized as colorless needles from ethyl acetate-petroleum ether (2.8 g), mp 149–50°. It gave a negative ferric reaction. (*Anal.* Found: C, 63.30; H, 5.90; Calc. for $C_{22}H_{26}O_8$: C, 63.15; H, 6.26%).

2-HYDROXY-3,4-DIETHOXY-3',4',5'-TRIMETHOXYDIBENZOYLMETHANE (**8**).—The above ester (**7**; 2.5 g) was converted into the β-diketone, 2-hydroxy-3,4-diethoxy-3',4',5'-trimethoxydibenzoylmethane (**8**) as described for **5**. It crystallized from ethyl acetate-petroleum ether as bright yellow needles (1.5 g), mp 101°. It gave a green ferric reaction. (*Anal.* Found: C, 62.80; H, 6.40; Calc. for $C_{22}H_{26}O_8$: C, 63.15; H, 6.26%).

7,8-DIETHOXY-3',4',5'-TRIMETHOXYFLAVONE (**2a**).—The above β-diketone (**8**; 1 g) was cyclized, as already described, to obtain the compound (**2a**) which crystallized from chloroform-petroleum ether as colorless needles (0.75 g), mp 184–85°: pmr δ 1.44 (m, 6H, 2X $-OCH_2CH_3$), 3.90 (s, 9H, 3X $-OCH_3$), 4.12–4.23 (m, 4H, 2X $-OCH_2CH_3$), 6.67 (s, 1H, C-3-H), 6.90 (d, 1H, J=8 Hz, C-6-H), 7.18 (s, 2H, C-2'-H & C-6'-H) and 7.80 (d, 1H, J=8 Hz, C-5-H). On direct comparison it was found to be different from the ethyl ether of prosogerin-E. (*Anal.* Found: C, 65.50; H, 5.90; Calc. for $C_{22}H_{24}O_7$: C, 65.99; H, 6.04%).

PATULITRIN.—Patulitrin crystallized from methanol as pale yellow needles (90 mg), mp 214–15°. It gave a positive Molisch's test for glycosides, red coloration with ammonium molybdate in acetic acid for ortho-dihydroxyls, and a brown ferric reaction; uv λ max (MeOH): 265 and 375 nm; $+AlCl_3$: 275 and 445 nm; $+AlCl_3$+HCl: 270 and 420 nm; +NaOAc: 265 and 390 nm; +NaOAc+H_3BO_3: 270 and 385 nm. On hydrolysis with alcoholic sulfuric acid (25 ml; 7%), it gave an aglycone, patuletin, which crystallized as yellow needles from methanol, mp 262–63°. The aqueous hydrolysate, when subjected to paper chromatography with *n*-butanol-pyridine-water (6:4:3), was found to contain glucose as the free sugar. This glucoside on complete methylation followed by acid hydrolysis yielded 3,5,6,3',4'-pentamethoxy-7-hydroxyflavone as colorless needles crystallized from methanol, mp 231°. On acetylation it gave patulitrin acetate which crystallized as colorless needles from ethyl acetate-petroleum ether, mp 148–49°.

RUTIN.—Rutin crystallized from methanol as pale yellow needles (100 mg), mp 190–91°. It gave a positive Molisch's test for glycosides, red coloration with Quastel reagent (7) and olive-green coloration with alcoholic ferric chloride; uv λ max (MeOH): 250 and 370 nm; $+AlCl_3$; 265 and 450 nm; $+AlCl_3$+HCl: 260 and 430 nm; +NaOAc: 260 and 380 nm; +NaOAc+H_3BO_3: 260 and 385 nm. On acid hydrolysis it gave quercetin as the aglycone which crystallized as pale-yellow needles from methanol, mp 316–17° and also yielded rhamnose and glucose as free sugars. On complete methylation followed by acid hydrolysis this glycoside yielded 3-hydroxy-5,7,3',4'-tetramethoxyflavone as colorless prisms from methanol.

ACKNOWLEDGMENTS

The authors wish to thank the Council of Scientific and Industrial Research, New Delhi and University Grants Commission, New Delhi, for financial assistances.

Received 13 June 1980.

LITERATURE CITED

1. R. N. Chopra, 1958. Indigenous Drugs of India, U. N. Dhur & Sons, Calcutta, India.
2. R. C. Sharma, A. Zaman and A. R. Kidwai, *Indian J. Chem.*, **2**, 83 (1964).
3. K. Jewers, M. J. Nagler, K. A. Zirvi, F. Amir and F. H. Cottee, *Pahlavi, Med. J.*, **5**(1), 1 (1974).
4. D. K. Bhardwaj, R. K. Jain, C. K. Mehta and G. C. Sharma, *Indian J. Chem.*, **16**B, 1133 (1978).
5. D. K. Bhardwaj, M. S. Bisht, C. K. Mehta and G. C. Sharma, *Phytochemistry*, **18**, 355 (1979).
6. D. K. Bhardwaj, M. S. Bisht, R. K. Jain and G. C. Sharma *Phytochemistry*, **19**, 1269 (1980).
7. J. H. Quastel, *Analyst*, **56**, 311 (1931).
8. T. J. Mabry, K. R. Markham and M. B. Thomas. The systematic identification of flavonoids, Springer Verlag, New York (1970).
9. R. K. Jain, Chemical studies of some naturally occurring polyphenolics and their derivatives, *Ph.D. Thesis*, University of Delhi, Delhi (India), (1978).
10. J. T. Marsh and H. Stephen, *J. Chem Soc.*, **127**, 1633 (1925).
11. W. Baker, *J. Chem. Soc.*, 1381 (1933).
12. H. S. Mahal and K. Venkataraman, *Curr. Sci.*, **2**, 214 (1933).
13. B. G. Doyle, F. Gogan, J. E. Gowan, J. Keane and T. S. Wheeler, *Sci. Proc. Royal. Dublin Soc.*, **24**, 291 (1948).
14. A. T. M. Dunne, J. R. Gowan, J. Keane, B. M. O. Keeley, D. O'Sullivan, M. M. Roche, P. M. Ryan and T. S. Wheeler, *J. Chem. Soc.*, 1252 (1950).