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SHORT COMMUNICATION

THE FLAVONOIDS OF THE LEAVES OF *ACACIA MEARNSII*

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Abstract—The leaves of *Acacia mearnsii* have been found to contain, in addition to four previously identified compounds (myricitrin, quercitrin, (+)-catechin and (+)-gallocatechin), a new flavonol glycoside for which the name mearnsitrin is proposed and the 3-glucosides of myricetin and quercetin.

INTRODUCTION

THE FLAVONOIDS present in *Acacia mearnsii* (black wattle) have long been studied, but with the exception of a limited examination of the leaves by Saayman and Roux,[1] these investigations have been confirmed to extracts obtained from either the bark, which is used commercially for the preparation of tanning extract, or the wood.[2–9] Saayman and Roux[1] isolated and identified myricitrin and quercitrin in the leaf and noted the presence of another pair of glycosides, one of which yielded myricetin on hydrolysis. (+)-Catechin and (+)-gallocatechin were identified by paper chromatography. In addition to myricitrin, quercitrin, (+)-catechin, and (+)-gallocatechin, the present investigation has resulted in the isolation and identification of the 3-glucosides of myricetin and quercetin (isoquercitrin), and a new flavonol glycoside (named mearnsitrin) which was found to be present in the leaves of about 8 per cent of trees grown from commercial seed.[10, 11] Fortunately, material was available from plant-breeding experiments which had produced families with up to 60 per cent of the trees containing the compound.

Myricetin-3-glucoside and isoquercitrin were isolated together as mixed crystals. In view of the very small yield obtained, no attempt was made to separate them, but the mixture was hydrolysed and myricetin and quercetin identified by paper chromatography and glucose by gas chromatography.

By its R_f values on paper chromatograms and reaction with various spray reagents, the new flavonol glycoside mearnsitrin was shown to have a phloroglucinol A ring and no free

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[10] F. C. J. ZEIJLEMAKER and A. M. MACKENZIE, *Rep. Wattle Res. Inst. for* 1965–1966, 57 (1966).
[11] A. M. MACKENZIE, *Tetrahedron Letters* 2519 (1967).

ortho-dihydroxy groups. Hydrolysis of the glycoside in dilute acid solution yielded a non-crystalline flavonol, mearnsetin, whose molecular formula was established as $C_{16}H_{12}O_8$ by high resolution mass spectrometry, and a sugar identified as rhamnose. Comparison of the u.v. spectra of the glycoside with its aglycone in ethanolic $AlCl_3$[12] established that the sugar residue was attached to the 3-position. Analysis of the trimethylsilyl derivative of the glycoside by NMR spectroscopy[13,14] whilst not giving much evidence regarding the structure of the flavonoid molecule because of considerable line broadening, did permit the establishment of a 1:1 mole ratio of sugar to aglycone.

Mearnsetin formed a crystalline acetyl derivative which, on examination by NMR, was found to contain five O-acetyl groups, one O-methyl group, one pair of *meta*-coupled and another pair of equivalent aromatic protons. This indicated a symmetrical substitution pattern in the B phenyl ring, i.e. most probably 3′,5′-dihydroxy-4′-methoxy. Demethylation of mearnsetin with pyridinium chloride[15] yielded myricetin. Final confirmation of the structure of mearnsetin as 4′-O-methylmyricetin (3,3′,5,5′,7-pentahydroxy-4′-methoxyflavone) was obtained with the identification of 4-O-methylgallic acid as a major product from KOH fusion under the conditions described by Roux.[16]

This evidence, together with that regarding the position of the rhamnose residue, defines the structure of mearnsitrin as 3′,5,5′,7-tetrahydroxy-4′-methoxyflavone-3-rhamnoside. Apart from the interesting genetic factors controlling its occurrence in black wattle,[10] mearnsitrin is the only methoxylated flavonoid found to date in wattle species and has not, to the best of the author's knowledge, been identified from any other source.

EXPERIMENTAL

NMR spectra were recorded by a Varian A60 spectrometer from $CDCl_3$ solutions containing tetramethylsilane as internal standard. Paper chromatograms were run on Whatman No. 1 paper using butan-1-ol, acetic acid, water (6:1:2 v/v), i.e. BAW, and 2% v/v acetic acid.

Extraction and Fractionation

2·25 kg of leaves, freshly picked from trees which had been shown by previous chromatographic examination to contain mearnsitrin,[10] were macerated with ethyl acetate in a 1-gal capacity Waring blendor and the resulting pulp left overnight under the solvent, agitated for 1·5 hr, and filtered. The residue was shaken with more ethyl acetate for a further 1·5 hr and filtered. The extract solutions were separately evaporated to dryness at 60° in a rotary evaporator to yield 94 and 33 g respectively of a sticky, dark-green residue which, combined and extracted overnight with petroleum ether (b.p. 40–50°), was obtained as a light-green, friable solid (84 g). Residual chlorophyl was removed by dissolving the solid in 200 ml warm methanol and pouring with vigorous stirring into 4 l water. 40 ml N HCl was added to flocculate the chlorophyll, which was filtered off. The brown filtrate was extracted four times with ethyl acetate to yield on evaporation, as before, 56 g of dry, friable solid.

This material was dissolved in a minimum of methanol and chromatographed on a 6 × 60 cm column of polyamide powder (Ultramid, B.A.S.F.), elution being carried out with methanol. 50-ml fractions were collected and examined by two-dimensional paper chromatography. The first compound eluted was mearnsitrin followed by (+)-catechin, myricitrin and quercitrin, (+)-gallocatechin, and, after prolonged elution, isoquercitrin and myricetin-3-glucoside. Considerable overlapping of compounds occurred and separation was obtained by streaking appropriate fractions on to Whatman 3MM paper, developing with the above-mentioned solvents, and eluting.

Myricitrin (*3′,4′,5,5′,7-pentahydroxyflavone-3-rhamnoside*) was identified by direct paper chromatographic comparison with authentic myricitrin in the previously mentioned solvents and in 75% aqueous phenol, by colour reactions and by u.v. spectra in alcohol alone and in the presence of $AlCl_3$. The i.r. spectrum (KBr)

[12] T. A. Geissman and L. Jurd, *Archs Biochem. Biophys.* **56**, 259 (1955)
[13] A. C. Waiss, Jr., R. E. Lundin and D. J. Stern, *Tetrahedron Letters* 513 (1964).
[14] T. J. Mabry, J. Kagan and H. Rosler, *Phytochem.* **4**, 177 (1965).
[15] J. B. Harborne, personal communication.
[16] D. G. Roux, *J. Am. Leather Chem. Assoc.* **53**, 384 (1958).

was not significantly different from that obtained by Drewes.[17] Hydrolysis yielded myricetin (identified by similar means) and rhamnose identified by gas chromatography of the TMS ether under conditions identical to those described by Kagan and Mabry.[18]

Quercitrin (*3′,4′,5,7-tetrahydroxyflavone-3-rhamnoside*) was identified in exactly the same manner as myricitrin.

(+)-*Catechin* ((+)-*3,3′,4′,5,7-pentahydroxyflavan*) was identified by paper chromatographic comparison with authentic (+)-catechin in four solvents (those mentioned above plus water-saturated ethyl acetate) and by colour reactions.

(+)-*Gallocatechin* ((+)-*3,3′,4′,5,5′,7-hexahydroxyflavan*) was identified in a similar manner to catechin except that chromatographic comparison with an ethyl acetate extract of dry golden wattle bark[19] resulted in intensification of the spot identified by comparison with photographs published by Maihs[20] as (+)-gallocatechin.

Mearnsitrin (*3′,5,5′,7-terahydroxy-4′-methoxyflavone-3-rhamnoside*). Despite numerous attempts this compound could not be crystallized. R_f-s were 0·80 (BAW), 0·35 (2% acetic acid) ((+)-catechin 0·63 and 0·34 respectively). Colour reactions were pale yellow in visible light deepening markedly on fuming with ammonia, dark brown in u.v. even when fumed with ammonia, no reaction with ammoniacal $AgNO_3$, bright yellow after spraying with toluene-*p*-sulphonic acid (visible and u.v.), and distinctly pinkish-brown developing slowly with *bis*-diazotized benzidine. U.v. spectral max. were at 264, 340 (EtOH), 275, 303, 340, 395 ($AlCl_3$), and 273, 372 nm (0·002 M NaOEt).

Mearnsetin. 100 mg glycoside yielded on acid hydrolysis a yellow aglycone (mearnsetin) and a sugar residue containing rhamnose only (identified by paper chromatography). The aglycone had R_f 0·81 (BAW) (quercetin 0·72, kaempferol 0·88). Colour reactions: yellow in visible light, bright yellow in u.v., same pinkish-brown as the glycoside with *bis*-diazotized benzidine, and again no reaction with ammoniacal silver nitrate. U.v. spectral max. were at 261, 366 (EtOH), 273, 308, 355, 427 ($AlCl_3$), 262, 368 (alcoholic $NaOAc/H_3BO_3$), and 276, 320 (shoulder), 410 nm, (0·002 M NaOEt 1 hr after mixing reagents). Penta-acetyl mearnsetin was prepared with pyridine and acetic anhydride. NMR examination revealed one methoxyl group, five acetyl groups, one pair *meta*-coupled protons ($J = 2{\cdot}4$ cps), and two equivalent aromatic protons. Mearnsetin (2 mg) was demethylated with excess of pyridinium chloride at 140° for 2 hr, giving myricetin and some unchanged mearnsetin. Mearnsetin (2 mg) was fused with KOH and the phenolic acid fraction isolated. Chromatographic comparison of this fraction established the identity of the major component as 4-*O*-methylgallic acid, obtained by acid hydrolysis of its methyl ester, prepared from gallic acid and dimethyl sulphate.[21]

Isoquercitrin (*3′,4′,5,7-tetrahydroxyflavone-3-glucoside*) and *myricetin-3-glucoside* (*3′,4′,5,5′,7-pentahydroxyflavone-3-glucoside*). These compounds, obtained as mixed crystals from alcohol/water, were found to have R_f 0·44 and 0·63 respectively in BAW and R_f 0·06 in 2% acetic acid. Colour reactions were the same as those obtained with quercitrin and myricitrin respectively. Acid hydrolysis gave glucose, quercetin and myricetin. Comparison of R_f values for the glycosides with those reported by Harborne[22] for quercetin-3-glycosides pointed to the compounds being the 3-glucosides of the respective flavonols.

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