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PHENOLIC NEORAUTANENIA ISOFLAVANOIDS

THE ISOLATION AND STRUCTURES OF NEORAUFLAVENE, (−)-NEORAUFLAVANE AND NEORAUFURANE, THREE NOVEL ISOFLAVANOIDS FROM *NEORAUTANENIA EDULIS*

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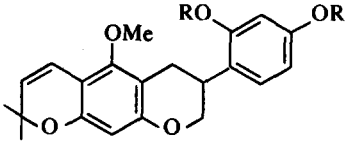
Abstract—The structures of the three novel isoflavanoïds obtained from *Neorautanenia edulis* has been established on the basis of physical methods and by conversions. One of these (−)-neorauflavene, represents the first natural isoflavene.

Extracts of the genus *Neorautanenia*, which frequently yield isoflavanoïds and compounds with related structure, are known to be toxic to fish.[1] Accordingly, the bark of *Neorautanenia edulis* was examined for phenolic constituents. We now report the isolation and structural elucidation of neorauflavene, neorauflavane and neoraufurane, of which the first represents a new class of isoflavanoïd compound that was successfully isolated for the first time from a natural source. Due to their exceptionally low concentrations they were characterized by physical methods and by selected conversions.

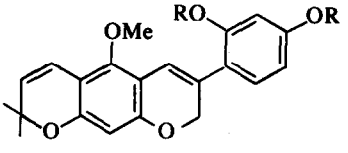
Both neorauflavane (**1**) and neorauflavene (**4**) were isolated as the dimethylethers, **2** and **5**, and the diacetates, **3** and **6**, and the molecular formulae of **2** and **5** established by high resolution mass spectrometry as $C_{23}H_{26}O_5$ and $C_{23}H_{24}O_5$ respectively.

The NMR data (Table) of the dimethylethers **2** and **5** and the diacetates **3** and **6** reveal the close relationship between these two compounds in so far that both have two OH groups, a 2,2-dimethylchromene ring and four aromatic protons with more or less corresponding chemical shifts. One of the latter protons appears as a singlet, while the coupling constants of the remaining three represent an ABX system. The values for the remaining uncoupled protons of **2** and **5** bear close resemblance to that of edulaan[2] (**7**) and the values, τ 3·78 and 3·79, are therefore assigned to the 8-protons present on the A rings.

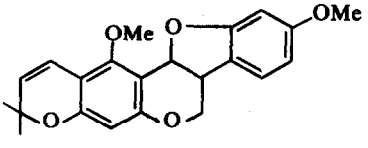
By comparison of the theoretical chemical shifts[3] for B-ring protons with the observed values for **2** we concluded that **2** should be the correct structure to account for the ABX system formed by the aromatic protons on ring B.



1; R = H
2; R = Me
3; R = COMe



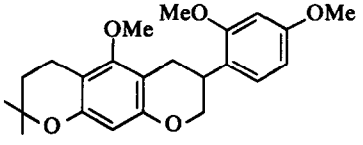
4; R = H
5; R = Me
6; R = COMe



7

As can be seen the similarities in chemical shifts (Table) are quite striking and these results provide further evidence for the general structural features present in both. At this stage it was clearly desirable to establish the relationship between **2** and **4**.

Indeed, when **2**, neorauflavane-dimethylether, and **5**, neorauflavene–dimethylether, were hydrogenated over Pd/C in ethylacetate a product, 2″, 2″ - dimethyl - 6, 7, 5″, 6″ - chromane - 5, 2′, 4′ - trimethoxyisoflavane (**8**), was obtained in both cases.

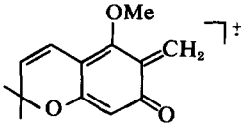


8

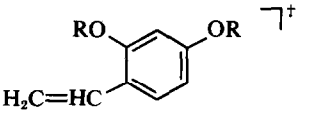
Conclusive proof for this structure was obtained when edulaan (**7**) was treated with HCl/MeOH and then methylated with $(CH_3)_2SO_4/K_2CO_3$/acetone to give neorauflavene-dimethylether (**5**). The position of the heterocyclic 4-proton was fully proved by correlation of the NMR data of **5** with that found for the synthetic isoflav-3-ene (**5**) obtained by the method of Bevan *et al.*[4]

The only other plausible structure, the isoflav-2-ene, was hereby eliminated. This was further supported by the fact that no retro-Diels–Alder fragmentation was observed.

The mass spectrum of the methoxylated isoflavan (**2**) agrees well with the established pattern for synthetic isoflavan compounds[5,6] on the assumption that ring A has a γ,γ-dimethylchromene group and ring B two methoxy substituents as shown in fragments **a** (*m/e* 218) and **b** (*m/e* 164). The fragment **c** in the mass spectrum of **3** suggests that both phenolic groups are present as substituents on the B ring.



a
m/e 218



b; R = Me *m/e* 164
c; R = COMe *m/e* 220

Confirmation was obtained by the observation that the only protons showing paramagnetic shifts on acetylation are those related to the ABX system for both **3** and **4**, and no perceptible shifts were observed for the heterocyclic protons nor the uncoupled 8-proton.

The absolute configuration of **3** and therefore of **1** as R follows from agreement between the ORD curve of the diacetate (**3**) of neorauflavane and that found by Clark–Lewis[7] for 3R-isoflavan compounds.

Neoraufurane (**9**) was also isolated as the dimethylether (**10**) and diacetate (**11**). The UV spectrum of X = **10** [λ_{max}^{EtOH} 234, 270, 296, 323, 337; log ε 4·09, 4·19, 4·10, 4·23 and 4·13] is characteristic of 2-phenylbensofurane compounds.[4,8,9] The molecular formula $C_{22}H_{24}O_5$ was established by high resolution mass-spectrometry for **10**.

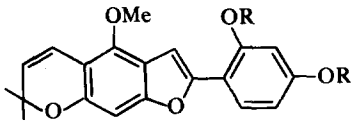
Table

Assignments of chemical shifts (τ in ppm) in the NMR spectra (*J* in c/sec)

Protons	Compound 2	3	5	6
8-H	3·78 (s)	3·75 (s)	3·79 (s)	3·75 (s)
3′-H	2·91 ($J_{3'-5'}$ = 3·0)	3·04 ($J_{3'-5'}$ = 3·0)	3·50 ($J_{3'-5'}$ = 3·0)	2·99 ($J_{3'-5'}$ = 3·0)
5′-H	3·49 ($J_{5'-6'}$ = 10)	2·95 ($J_{5'-6'}$ = 10·0)	3·47 ($J_{5'-6'}$ = 10·0)	2·97 ($J_{5'-6'}$ = 10·0)
6′-H	3·53	2·70	2·72	2·57
3″-H	4·48 (d) ($J_{3''-4''}$ = 10)	4·45 (d) ($J_{3''-4''}$ = 10·0)	4·62 (d) ($J_{3''-4''}$ = 10·0)	4·43 (d) ($J_{3''-4''}$ = 10·0)
4″-H	3·42 (d)	3·40 (d)	3·44 (d)	3·43 (d)
CH_3 CH_3	8·56 (6H)	8·54 (6H)	8·57 (6H)	8·57 (6H)
5-OCH_3	6·25 (6H)	6·25 (3H)	6·19 (6H)	6·15 (3H)
4′-OCH_3	6·25	—	6·19	—
6′-OCH_3	6·18 (3H)	—	6·27 (3H)	—
$COCH_3$	—	7·69 (3H)	—	7·67 (3H)
$COCH_3$	—	7·72 (3H)	—	7·69 (3H)
2-H	5·67 (q) (1H) (J_{2ax-3} = 10; J_{2eq-3} = 3·5) 6·00 (t) (1H) ($J_{2ax-2eq}$ = 10)	5·67 (q) (1H) (J_{2ax-3} = 10; J_{2eq-3} = 3·5) 6·04 (t) (1H) ($J_{2ax-2eq}$ = 10·0)	5·25 (s) (2H)	5·09 (s) (2H)
3-H	6·20–6·56 (m) (1H)	6·20–6·56 (m) (1H)	—	—
4-H	7·06–7·23 (bs)(1H)	7·05–7·20 (bs) (1H)	3·25 (s) (1H)	3·17 (s) (1H)

s = singlet; d = doublet; t = triplet; m = multiplet; bs = broad singlet.

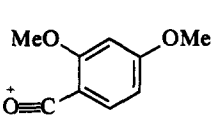
The presence of one –OMe [6·24 (3H)] and two acetoxy groups [7·64 (3H) and 7·65 (3H)] in the NMR spectrum of **11** indicate the presence of two OH groups in **9**, while the chemical shifts at τ 6·15 (3H), 6·04 (3H) and 5·89 (3H) (Table), thus three –OMe groups for **10**, support this.



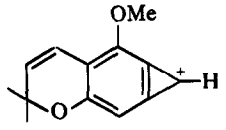
9; R = H
10; R = Me
11; R = COMe

Further structural features revealed by the NMR spectrum of **10** are the presence of 2,2-dimethylchromene group [τ = 4·40 (1H) (d), 2·23 (1H) (d) and 8·56 (6H) (s)] and five aromatic protons which are assigned as follows: two para coupled singlets at τ 2·72 and 3·45 corresponding to the 3- and 7-protons, while the other three give rise to an ABX system with τ = 2·09 (J = 10) and 3·36 (J_1 = 10; J_2 = 3·5) showing ortho coupling, while the coupling constant J_2 = 3·5 indicates a meta coupling between the protons at τ 3·36 and 3·30 (J_2 = 3·5).

Comparison of the NMR data of **2**, **5** and **9** correlate their substitution patterns as regards the OMe and 2,2-dimethylchromene groupings, while the paramagnetic shifts of the aromatic protons after acetylation support the substitution pattern of structure **9**. Further support for the phenylbensofurane structure was found in the MS results.[10] Not only was this in accordance with the expected for **10** but was of great significance to prove the substitution pattern as illustrated in fragments **d** and **e**.



d (*m/e* 165)



e (*m/e* 201)

EXPERIMENTAL

M.ps were determined on a Kofler-hotstage apparatus and were uncorrected. Mass spectra were measured with an AEI-MS-9 spectrometer with direct insertion technique. NMR spectra were recorded on a Varian Ha-100 or T-60 instrument with TMS as internal standard (τ = 10·00) in $CDCl_3$. UV refers to a soln in spectroscopically pure EtOH and were recorded on a Perkin-Elmer 402 spectrophotometer while ORD measurement was done on a Jasco ORD/UV-5-polarimeter.

Extraction of **1**, **4** *and* **9**. The following procedure, as applied to Neorautanenia material exemplifies the method used for large-scale extractions. Dried, milled bark (5 mg) was extracted with diethyl ether and the extract concentrated to a brown syrup (45 g) and chromatographed on silica-gel to give a phenolic fraction (2·3 g) containing **1**, **4** and **9**.

Isolation of **2**, **5** *and* **10**. Half of the mixture (1·02 g) was dissolved in dry acetone (100 ml) and anhyd K_2CO_3 (4·0 g) added. Me_2SO_4 (3·0 ml) was added dropwise to the mixture over a period of 60 min at a temp of 50° with stirring. Evaporation of the acetone filtrate afforded an oily residue to which a soln of 5% KOH (10 ml) was added. After the addition of water (100 ml) the mixture was extracted with benzene (3 × 50 ml). The extract was washed with water, dried ($MgSO_4$) and filtered. The solvent was evaporated and the residue separated on prep TLC plates [silica-gel GF_{254}–benzene:hexane (3:2)] in three bands with R_f 0·44, 0·30 and 0·15 resp from which the compounds were extracted as normal.

The first band yielded **2** which crystallised from benzene:hexane (9:1) as colourless needles, m.p. 132·9°C, $[\alpha]_D^{22} = -7·78°$ (c = 0·2 in $CHCl_3$). (Found: M^+ 382·177198. $C_{23}H_{26}O_5$ requires: 382·178012), *m/e* 382 (37·2), 367 (100), 219 (3·6), 218 (4·3), 203 (15·0), 191 (11·1), 164 (17·3), 163 (3·6), 151 (33·8), 149 (40·0) and 121 (16·6).

Compound **5** was isolated from the second band as a colourless oil. (Found: M^+ 380·162012, $C_{23}H_{24}O_5$ requires: 380·162362), *m/e* 380 (50·3), 365 (100), 350 (12·0), 349 (8·5), 335 (23·7), 334 (3·5), 319 (5·5), 3·7 (70), 291 (8·5), 263 (8·5), 203 (11·0), 189 (23·6), 176 (10·4) and 161 (15·1).

From the third band was obtained neoraufuranedimethylether also as a colourless oil. (Found: M^+ 366·147208. $C_{22}H_{22}O_5$ requires: 366·146713), *m/e* 366 (84·0), 351 (100), 336 (20·2), 321 (9·0), 306 (7·6), 201 (7·4), 189 (22·4), 189 (6·4), 171 (17·3), 168 (38·3), 165 (28·2), 161 (14·9) and 137 (27·8).

Isolation of **3**, **6** *and* **11**. The other half (1·02 g) of the phenolic mixture was taken up in dry pyridine (7·0 ml) and Ac_2O (9·0 ml) was added. The mixture was refluxed for 30 min on a waterbath. The soln was then poured into ice water (50 ml) and kept at 0° for 30 min. The light brown ppt was extracted with benzene (4 × 50 ml) and this with 3N HCl (4 × 25 ml) and distilled water. The extract was dried ($MgSO_4$) and after filtration and evaporation of the solvent the mixture was separated with prep TLC [silica-gel GF_{254}–benzene:hexane:acetone (5:4:1)] and gave (i) neorauflavane-diacetate (**3**) m.p. 137·2° from benzene. (Found 438·168012. $C_{25}H_{26}O_7$ requires 438·167839); (ii) neorauflavene-diacetate (**11**), colourless oil. (Found 436·152310. $C_{25}H_{24}O_7$ requires 436·152190), and (iii) neoraufurane-diacetate (**11**) as a colourless oil. (Found 422·135946. $C_{24}H_{22}O_7$ requires 422·136541).

2″, 2″ - Dimethyl - 6, 7, 5″, 6″ - chromane - 5, 2′, 4′-trimethoxy - isoflavane (**8**). Compound **7** (30 mg) was hydrogenated in EtOAc:AcOH (1:1) (9 ml) containing 10% Pd/C at 60°C for 5 h at 6 atm. After absorption the catalyst and solvent were removed to give a hydroxy compound that was, due to instability, directly converted to **7** as usual. (Found 384·193830. $C_{23}H_{28}O_5$ requires 384·193660).

Hydrogenation of **2**. A soln of **2** (10 mg) in EtOAc was hydrogenated at room temp using 20 mg of 2% Pd/C catalyst. After 30 min the catalyst was filtered off and a product, identical in all aspects (MS, NMR and R_f), to **8** was obtained.

Hydrogenation of **5**. Using the same procedure as described, the hydrogenation of **5** afforded also compound **8**.

Preparation of **5** *from* **7**. Edulaan **7** (200 mg) in EtOH (12 ml) and conc HCl (0·25 ml) was refluxed for 10 min. The mixture was cooled down, water added (150 ml) and extracted with ether (3 × 50 ml). The combined ether extracts were washed with 5% $NaHCO_3$-soln (3 × 50 ml), dis-

tilled water (2 × 20 ml) and dried (Na_2SO_4) to give **4** that was immediately methylated as usual to give **4**, identified by MS, NMR, and R_f.

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