295. *The Extractives of* Neorautanenia pseudopachyrrhiza*: the Isolation and Structure of a New Rotenoid and Two New Isoflavanones.**

By L. Crombie and D. A. Whiting.

The root of the leguminous plant *N. pseudopachyrrhiza* contains pachyrrhizin (I) and three new compounds neotenone, dolineone, and nepseudin. The first of these is shown by degradation and spectral examination to be the methoxymethylenedioxyfuranoisoflavanone (II); dolineone is the methylenedioxyrotenoid (XV) corresponding to it in structure. Nepseudin is an isoflavanone similar to neotenone but with a unique 2′,3′,4′-trimethoxylated ring A attachment replacing the normal 2′,4′,5′-oxygenation pattern. Neotenone and dolineone, as well as dehydroneotenone, are shown also to be present in *Pachyrrhizus erosus*. The unformulated compounds neorautone and neorautenone reported in *N. edulis* are shown to be pachyrrhizin and neotenone, respectively. The *Pachyrrhizus* and two *Neorautanenia* species are closely related and the compounds contained in them (I), (II), (III), (XV), (XVIII), (XXI). (XXII), and (XXIII), constitute an unusually extended pattern of variation on a basic furanoisoflavanoid theme and raise interesting questions of biogenetic connexion.

The root of *Neorautanenia pseudopachyrrhiza* Harms. (*Dolichos pseudopachyrizus*) is recorded as being toxic to insects,[1] and this, and its familial relationships, led us to suspect that it might be a source of new rotenoids or their close relatives. Supplies of root were obtained for us from Tanganyika by the Tropical Products Institute and, on examination of the methylene chloride extract by chromatography and counter-current distribution, four crystalline components were isolated. These were a yellow-green compound, m. p. 209°, and three colourless compounds, m. p. 116°, 180·5°, and 235°, respectively. A preliminary examination of the yellow-green compound, $C_{19}H_{12}O_6$, showed its reactions to be closely similar to those of pachyrrhizin (I)[2,3] and its identity was established by comparison with an authentic specimen isolated from *Pachyrrhizus erosus* (yam beans). The three other compounds are new and their reactions and structures are dealt with in this paper. Observations are also made on the nature of certain previously unidentified compounds from related plant sources. It is now apparent that a comprehensive pattern of variation on a common furanoisoflavanoid structural theme is to be found in the Phaseolea tribe of the sub-family Papilionatae to which these plants belong; the biosyntheses and biogenetic connexions of the compounds should be of unusual interest.

The compound, m. p. 180·5°, which we named neotenone, had the formula $C_{19}H_{14}O_6$. It was optically inactive, contained one methoxyl group, and gave a positive Labat test [4]

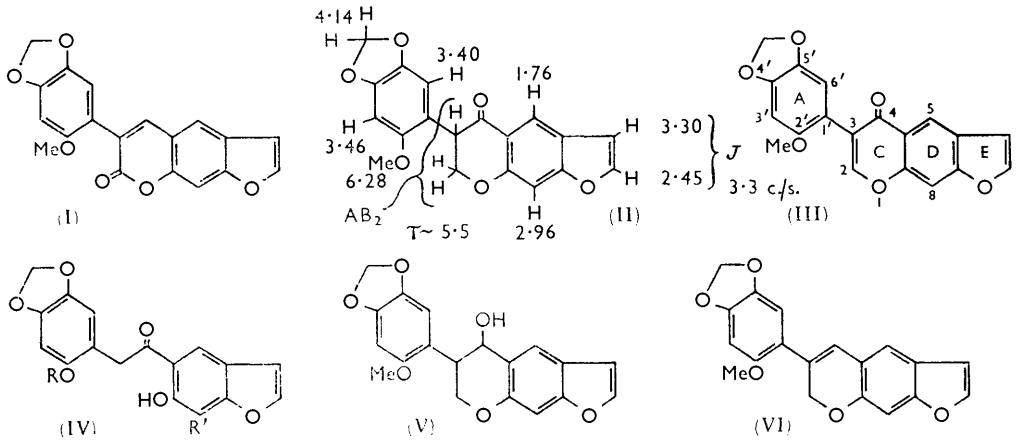
* For preliminary information see Crombie and Whiting, *Tetrahedron Letters*, 1962, No. 18, p. 801.

[1] H. J. Holman, " A Survey of Insecticide Materials of Vegetable Origin," The Imperial Institute, London, 1940.
[2] Norton and Hansberry, *J. Amer. Chem. Soc.*, 1945, **67**, 1609.
[3] Simonitsch, Frei, and Schmid, *Monatsh.*, 1957, **88**, 541.
[4] Labat, *Bull. Soc. Chim. biol.*, 1933, **15**, 1344.

for a methylenedioxy-group. A purple coloration in the Durham test [5] and a green one in the Rogers and Calamari test [6] indicated that the compound might be a rotenoid. The infrared and ultraviolet data were strongly reminiscent of the rotenoid elliptone,[7] and, as with a rotenoid, manganese dioxide [8] in refluxing acetone yielded a dehydro-compound, the carbonyl frequency of neotenone (1684 cm.$^{-1}$) being depressed to 1658 cm.$^{-1}$ as expected. Despite these preliminary indications, however, neotenone proved to be, not a rotenoid, but a new isoflavanone (II), the dehydro-compound having structure (III).

On alkaline hydrolysis dehydroneotenone yielded the deoxybenzoin (IV; R = Me, R′ = H) and formic acid. This establishes the isoflavone character of the dehydro-compound (III), and the parent isoflavanone was re-synthesised from the deoxybenzoin (IV; R = Me, R′ = H). The latter was treated with ethyl orthoformate, pyridine, and piperidine [9] to give the ketone (III) which when reduced with potassium borohydride gave the isoflavanol (V), presumably a product of 1,4-reduction followed by further reduction of the resulting ketone. This alcohol (V) was identical with a specimen made by reducing neotenone itself with borohydride. On dehydration with phosphorus oxychloride and pyridine the fully conjugated compound (VI) was obtained and on oxidation with chromic oxide the alcohol was converted into neotenone.

The full structure of the dehydro-compound (III) was derived by oxidation with alkaline hydrogen peroxide. This gave a mixture of two acids which could be separated. The first was the methylenedioxy-acid (VII) which was identical with an authentic

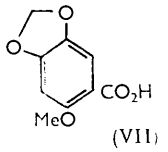


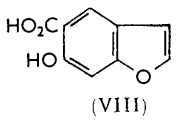
specimen: [10,11] the second gave the typical blue ferric colour of a salicylic acid [7] which, from spectroscopic, analytical, and other considerations had a fused furan attachment. There are six possibilities for this attachment, and to aid identification the assumption was made, on biogenetic grounds, that the oxygenation of the aromatic ring was probably resorcinolic in type. The linear fusion (II) would then give the acid (VIII) as the degradation product whilst the angular fusion (IX; cf. II) would give (X). The benzofuran (X) is a known product of the degradation of elliptone and has been synthesised.[7] It was not identical with the degradation product from neotenone.

The other acid (VIII) was therefore made from the hydroxycoumaranone (XI), prepared by base-catalysed cyclisation of the product of the Hoesch reaction between resorcinol and

[5] Jones and Smith, *Ind. Eng. Chem., Analyt.*, 1933, **5**, 75.
[6] Rogers and Calamari, *Ind. Eng. Chem., Analyt.*, 1936, **8**, 135.
[7] Harper, *J.*, 1939, 1099, 1424; 1942, 587, 593.
[8] Crombie, Godin, Whiting, and Siddalingaiah, *J.*, 1961, 2876.
[9] Warburton, *Quart. Rev.*, 1954, **8**, 67.
[10] Campbell, Hopper, and Campbell, *J. Org. Chem.*, 1951, **16**, 1736.
[11] Arnold and Bortnick, *J. Amer. Chem. Soc.*, 1945, **67**, 1798.

chloroacetonitrile.[12] This was converted by Huang-Minlon reduction into the phenol [13] (XII; R = H) and carboxylated either directly by the Kolbe reaction or indirectly *via*


(VII)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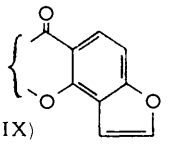
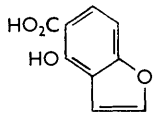
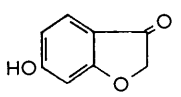
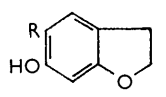

(IX)


(X)

the Gattermann synthesis and silver oxide oxidation to give the salicylic acid (XII; R = CO_2H).[14] The ester of this was dehydrogenated with palladium and hydrolysed to give the acid (VIII),[15] identical with that obtained by the oxidative degradation. Chemical evidence for the structure of neotenone is thus complete and the results from the proton magnetic resonance spectrum [16] are in full agreement. The assignments are shown in cipher (II): the four benzenoid protons show no spin-coupling, as required by (II), but not by (IX) for which a quartet with J ~9 cycles/sec. would be observed for the adjacent protons. Two protons at τ 4·14 are characteristic of the methylenedioxy-group and the spin-coupled protons at 2·45 and 3·30 (J 3·3 c./sec.) confirm the presence of the furan. Neotenone was always isolated in the (±)-form, even though nothing more alkaline than Woelm " neutral " alumina was used in the isolation.

The third compound from *N. pseudopachyrrhiza*, m. p. 235°, was named dolineone. [This was called dolichone in our preliminary communication but because of possible confusion with the terpenoid alcohol dolichol we have changed two letters in the name (we thank Dr. R. Brettle for his comments on the trivial names).] The amount of dolineone available was only about 100 mg., so experiments had to be chosen carefully. Its molecular

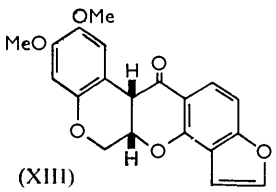

(XI)


(XII)

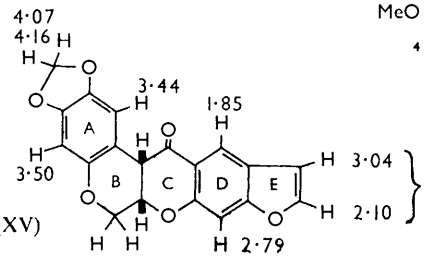
formula was $C_{19}H_{12}O_6$ and the mass-spectral molecular weight determined by Dr. R. I. Reed and Dr. J. M. Wilson of the University of Glasgow was in exact agreement with this. The ultraviolet spectrum of dolineone closely resembled that of neotenone (II) and elliptone (XIII) (Figure) and suggested a generally similar structure with a benzofuran system [contrast the ultraviolet spectrum of rotenone (XIV)]. Infrared data bear out the resemblance. Dolineone gave a green-blue Rogers and Calamari test but no Durham reaction: a methylenedioxy-group was present according to the blue Labat test. The proton magnetic resonance spectrum was found to be interpretable on the basis of structure (XV), as shown, and this structure is also in agreement with the remainder of the information above.

In the light of this, two degradations were carried out with the remaining material in order to test the proposal. The first was designed to establish the presence of the 12,12a,6a,6,5 sequence of carbon and oxygen atoms. The second was designed to show the presence of the furanodeoxybenzoin fragment (IV; R = R′ = H). Dolineone (ketone, $\nu_{max.}$ 1684 cm.$^{-1}$; cf. rotenone,[17] $\nu_{max.}$ 1674 cm.$^{-1}$) was dehydrogenated with manganese dioxide to give 6a,12a-dehydrodolineone (XVI) with a characteristic fall of the carbonyl frequency to 1639 cm.$^{-1}$ (cf. 6a,12a-dehydrorotenone,[8] $\nu_{max.}$ 1634 cm.$^{-1}$; 6a,12a-dehydropachyrrhizone, $\nu_{max.}$ 1646 cm.$^{-1}$). The dehydro-compound was then further oxidised at C-6 with lead tetra-acetate, and the whole crude product now showed a lactone band at

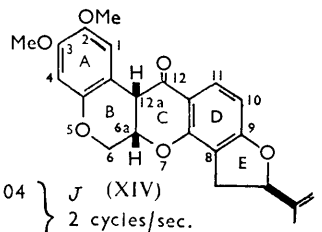
[12] Sonn, *Ber.*, 1917, **50**, 1262.
[13] Davies, McCrea, Norris, and Ramage, *J.*, 1950, 3206.
[14] Eisenbeiss and Schmid, *Helv. Chim. Acta*, 1959, **42**, 61.
[15] Reichstein, Oppenauer, Grüssner, Hirt, Rhyner, and Glatthaar, *Helv. Chim. Acta*, 1935, **18**, 816.
[16] Crombie and Lown, *J.*, 1962, 775.
[17] Büchi, Crombie, Godin, Kaltenbronn, Siddalingaiah, and Whiting, *J.*, 1961, 2843.



(XIII)



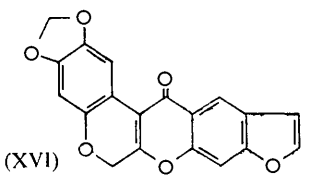
(XV)



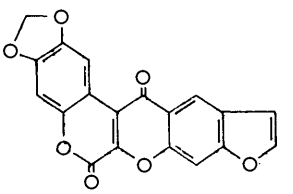
(XIV)

J 2 cycles/sec.

1748 cm.$^{-1}$ with a 12-ketone band at 1658 cm.$^{-1}$ (cf. rotenonone,[8] 1730 and 1645 cm.$^{-1}$; pachyrrhizonone, 1742 and 1660 cm.$^{-1}$), thus accounting for the presence of atoms 12, 12a, 6a, 6, and 5 as in (XVII). A small specimen of dolineone was then treated with zinc dust and alkali [8,18,19] to give the deoxybenzoin (IV; R = R′ = H). The unchelated hydroxyl



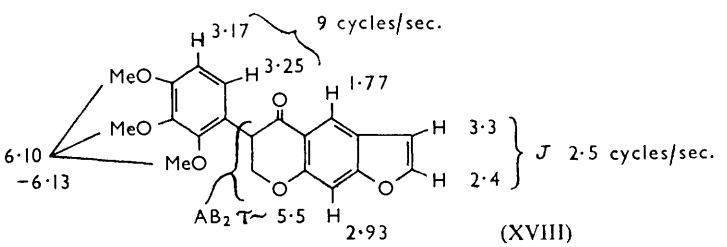
(XVI)



(XVII)

group was methylated and the monomethyl ether was purified by thin-layer chromatography. The isolated material gave the same three colour reactions as the methoxylated deoxybenzoin (IV; R = Me, R′ = H) obtained from neotenone, and was inseparable from it in mixed chromatograms with three different solvent systems.

In addition to this information, Dr. Reed and Dr. Wilson report that the mass-spectral fragmentation pattern is fully in agreement with structure (XV). The nuclear magnetic resonance spectrum indicates that the B/C fusion in (XV) is *cis* as the 1-hydrogen atom is located at τ 3·44 and is therefore not being strongly negatively shielded by the carbonyl group.[16,20] Professor C. Djerassi has kindly informed us that dolineone, like rotenone, has a positive Cotton effect (first extremum near 362 mμ).[21] It is therefore highly likely that the *cis*-B/C fusion has the same absolute configuration as rotenone (XIV).[17]

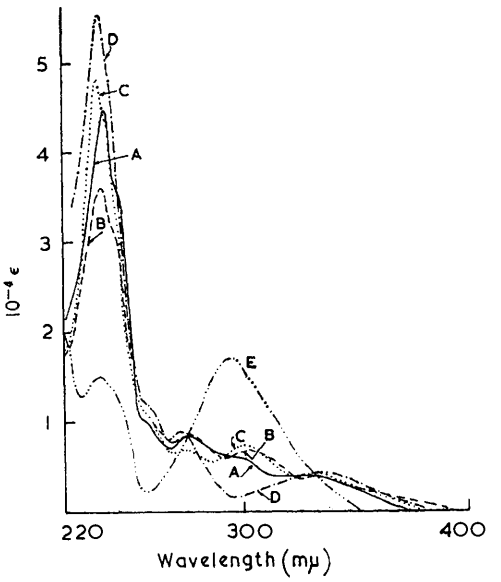


(XVIII)

The fourth compound from *N. pseudopachyrrhiza* was named nepseudin and had m. p. 116°. It gave an ambiguous greenish Labat test, a negative Durham reaction, and a strong Rogers and Calamari test. Analyses indicated a formula $C_{20}H_{18}O_6$, the compound containing three methoxy-groups. In the infrared spectrum the ketone frequency (1686 cm.$^{-1}$) suggested that nepseudin might be a rotenoid or an isoflavanone, particularly

[18] Butenandt, *Annalen*, 1928, **464**, 253.
[19] LaForge and Smith, *J. Amer. Chem. Soc.*, 1929, **51**, 2574.
[20] Crombie and Lown, *Proc. Chem. Soc.*, 1961, 299.
[21] Djerassi, Ollis, and Russell, *J.*, 1961, 1448.

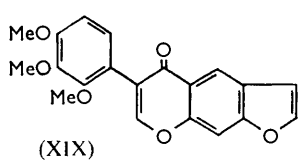
since dehydrogenation gave a dehydro-compound with unsaturated ketonic absorption at 1647 cm.$^{-1}$ (mull). The kinship of nepseudin to neotenone and dolineone is shown by the great similarity (Figure) of their ultraviolet spectra (and those of their dehydro-derivatives). With the above information in mind, the nuclear magnetic resonance spectrum of nepseudin could be interpreted in terms of (XVIII), the assignments being as shown. The proton integral showed three methoxyl groups and the presence of the furan ring was indicated



Ultraviolet absorption of (A) elliptone, (B) dolineone, (C) neotenone, (D) nepseudin, and (E) rotenone.

by the characteristic spin-coupled protons shown. No methylenedioxy-group was present and two of the four aromatic protons were adjacent and spin-coupled.

On hydrolysis, the dehydro-compound (XIX) gave the deoxybenzoin (XX) and not a derrisic acid type of compound, thus showing nepseudin to be an isoflavanone and not a rotenoid. The structure of the dehydro-compound was rigorously proved by oxidation with alkaline hydrogen peroxide, the benzofuran acid (VIII) and 2,3,4-trimethoxybenzoic acid,[22] both identical with synthetic specimens, being obtained. As in the case of neotenone, only (±)-nepseudin was encountered. The oxygenation pattern 2′,3′,4′, instead of the usual 2′,4′,5′, is unique in ring A of the isoflavanone and rotenoid series.



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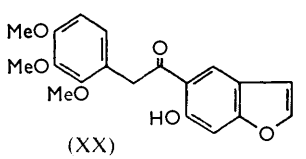
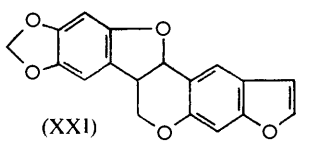


(XX)



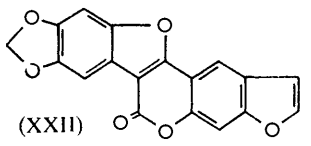
(XXI)

The work on *Neorautanenia pseudopachyrrhiza* can now be considered in the context of a recently reported investigation of *Neorautanenia edulis* by Van Duuren,[23,24] and of the investigations of *Pachyrrhizus erosus* (yam beans) by Norton and Hansberry,[2] Meijer,[25] and Schmid and his colleagues.[3,14,26] All three plants are members of the tribe Phaseoleae of the sub-family Papilionatae of the Leguminosae. Van Duuren has reported the

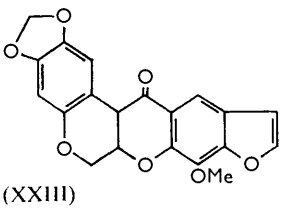
[22] Will, *Ber.*, 1888, **21**, 2020.
[23] Van Duuren, *J. Org. Chem.*, 1961, **26**, 5013.
[24] Van Duuren and Groenewoud, *J.S. African Chem. Inst.*, 1950, **3**(2), 29, 35.
[25] Meijer, *Rev. Trav. chim.*, 1946, **65**, 835.
[26] Bickel and Schmid, *Helv. Chim. Acta*, 1953, **36**, 664.

isolation of seven compounds from *N. edulis*, but only one of these, neodulin (edulin *), (XXI) has so far been formulated. Consideration of the reported properties of two others of the seven, neorautone (" substance 1 ") and neorautenone (" substance 3 "), led us to the view that, despite analytical discrepancies and the report of no methoxyl in the latter,[23] neorautone should be identical with pachyrrhizin, and neorautenone with (±)-neotenone. Through the kindness of Dr. Van Duuren in supplying samples for comparison we have established that this is so.

P. erosus, as examined by Norton and Hansberry,[2] yielded six crystalline compounds. One of these was rotenone, and the structures of three others were determined by Schmid and his colleagues. These were pachyrrhizin (" compound III ") (I),[3] erosnin (" compound I ") (XXII),[14] and the rotenoid pachyrrhizone (" compound II ") (XXIII).[26]



(XXII)



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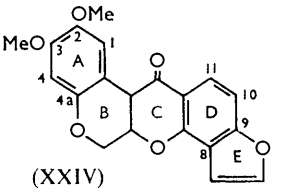

(XXIV)

" Compound VI " of Norton and Hansberry [2] had m. p. 242°. We noted that this was the same m. p. as our dehydroneotenone (III), and the analytical figures [2] were in much better agreement with $C_{19}H_{12}O_6$ than $C_{20}H_{12}O_6$ as proposed by them. On treatment with alkali a compound with a double m. p. 148° and 164° was said to be obtained and our deoxybenzoin (IV; R = Me, R′ = H) had m. p. 162—163°. Identification of " compound VI " as dehydroneotenone thus seemed likely. Dr. W. D. Ollis and Dr. K. Robinson have informed us that they have re-isolated " compound VI " and independently arrived at the view that it has structure (III). This is confirmed by direct comparison (mixed m. p. and spectra) of our dehydroneotenone and their sample of " compound VI " from *P. erosus*. In collaboration with Dr. P. J. Godin we have also isolated dolineone (XV) from *P. erosus* seeds, and thin-layer chromatography has shown that neotenone is also present. One other substance, " compound V " or erosone,[2] deserves mention. This component of *P. erosus* was reported by Norton and Hansberry to be a rotenoid, probably an isomer (not a stereoisomer) of elliptone which has structure (XXIV).[7] In view of the context of its occurrence, a linear D/E fusion with oxygenation in ring D still at C-9 (as in dolineone) seems an obvious possibility. But since nepseudin (XVIII) occurs in this sub-family it seems unwise to conclude that ring A must have the same 2,3,4a ring A oxygenation of elliptone and rotenone. It could be 3,4,4a.

Within these three closely related plants there thus occurs a remarkably rich pattern of variation on a basic isoflavanoid structural theme. The matter is summarised in Table 1 and it is almost certain that for any of the three plants the list of related compounds is

TABLE 1.

Occurrence of related isoflavanoids and rotenoids.

Structure	(I)	(II)	(III)	(XIV)	(XV)	(XVIII)	(XXI)	(XXII)	(XXIII)
N. pseudopachyrrhizus ...	+	+			+	+			
N. edulis	+	+					+		
P. erosus	+	+	+	+	+			+	+

incomplete. This is particularly so since severe seasonal fluctuations in the proportions of

* The name eduline has already been used for an alkaloid [27] and Dr. Van Duuren agrees that the name edulin used for compound [23] (XXI) should be replaced by neodulin.[28]

[27] Iriarte, Kincl, Rosenkranz, and Sondheimer, *J.*, 1956, 4170.
[28] Van Duuren, personal communication.

the components are known to occur in *N. edulis*,[24] and we have observed variations in batches of *N. pseudopachyrrhiza* and even in *P. erosus* seeds (though in the last case it may be due to minor varietal differences). This means in practice that components readily isolated from material gathered at one season are easily overlooked in batches gathered at another. There seems little doubt that interesting biogenetic connexions will emerge here, and *N. pachyrrhizus* is the first plant in which both an isoflavanone and its corresponding rotenoid have been shown to occur together. (It is probable that the corresponding isoflavone occurs with α-toxicarol in *Derris malaccensis* [29] though its structure was never finally established.) Thus far we have not encountered flavanoids corresponding to the compounds under consideration, although biosynthetic connexions are formally possible.[30]

Experimental

Except where stated otherwise, the following apply. Ultraviolet spectra were determined for ethanol solutions: log ε in parentheses follows $\lambda_{max.}$ and i denotes an inflexion. Infrared spectra were determined for chloroform solutions with sodium chloride optics. Molecular weights were determined ebullioscopically in benzene or chlorobenzene. In chromatographic work the letters N, A, or K together with a numeral refer to neutral, acid, or alkaline alumina of the numbered Brockmann grade. Evaporation signifies evaporation under reduced pressure, and drying refers to the use of anhydrous sodium sulphate.

Extraction of Neorautanenia pseudopachyrrhiza.—Crushed dried root (4 kg.) was repeatedly macerated with light petroleum (b. p. 40—60°) and then with methylene chloride. The methylene chloride extracts were distilled under reduced pressure and the residual brown gum (65 g.) was dissolved in warm benzene (200 ml.), which was then filtered and cooled. Two-thirds of this solution was chromatographed on Woelm alumina (N3) (1280 g.) and eluted with benzene. The early fractions gave a brownish wax and there followed fractions which on evaporation and treatment with methanol crystallised, to give crude (±)-neotenone (2·9 g.). Continued elution then gave pachyrrhizin (50 mg.). The mother-liquors from various fractions which yielded neotenone were then re-chromatographed on alumina (N3). The first eluted material yielded dolineone (3 mg.) and this was followed by nepseudin (480 mg.). A second and similar treatment of the methylene chloride extract of 5 kg. of bark (same batch) gave crude nepseudin (1·69 g.), yielding pure material (0·875 g.). Working-up a batch of bark (8 kg.) from the same source but received at a different time of the year gave crude dolineone (100 mg.) from the mother-liquors of the chromatogram fraction which had yielded pachyrrhizin. Counter-current distribution has been helpful in obtaining further crystalline material from intractable gum. Thus a brown gum (0·6 g.) obtained in the first extraction above, from which nothing further could be crystallised, was partitioned in a Craig apparatus by a 60 transfer fundamental procedure and an equilibrated solvent system of methanol (2 l.), water (0·5 l.), nitromethane (0·5 l.), light petroleum (b. p. 40—60°; 2·66 l.), and carbon tetrachloride (0·33 l.). Evaporation of tubes 3—6 gave pachyrrhizin (5 mg.), m. p. 208° (from methanol), and tubes 9—14 gave, after two crystallisations from methanol, nepseudin (10 mg.), m. p. 114—115°.

Neotenone, crystallised from methanol or chloroform–methanol, had m. p. 180—180·5°, $[\alpha]_D^{21}$ 0° (in benzene or chloroform or 1 : 1 chloroform–methanol), $\lambda_{max.}$ 235 (4·68), 275 (3·83), 300 (3·87) and 335 (3·61) mμ, $\nu_{max.}$ 1684 (C=O), 1623, 1582, 1541 (aryl) cm.$^{-1}$ (Found: C, 67·75; 67·25; 67·65; H, 3·95, 4·25, 4·4; OMe, 9·5%; *M*, 312. $C_{18}H_{11}O_5$·OMe requires C, 67·45; H, 4·15; OMe 9·2%; *M*, 338).

Pachyrrhizin crystallised from methanol in yellow-green needles, m. p. and mixed m. p. 207—209° with a specimen isolated by us from *P. erosus*, and with a specimen kindly presented to us by Professor H. Schmid (Found: C, 68·05; H, 3·85%; *M*, 343. Calc. for $C_{19}H_{12}O_6$: C, 67·85; H, 3·6%; *M*, 336).

(+)-*Dolineone* crystallised from methanol in needles m.p. 233—235°, $[\alpha]_D^{20}$ +135° (*c* 1·3 in $CHCl_3$), $\lambda_{max.}$ 237 (4·56), 275 (3·84), 305 (3·71), and 335 (3·50 mμ), $\nu_{max.}$ 1684 (C=O), 1629, 1587, 1546, 1504 (aryl) cm.$^{-1}$, $\nu_{max.}$ (mull) 1686, 1631, 1587, 1546, 1504, and 937 cm.$^{-1}$ (Found: C, 67·75, 67·5; H, 3·65, 3·85. $C_{19}H_{12}O_6$ requires C, 67·85; H, 3·6%). The 3 mg. specimen isolated above had m. p. 249° although its infrared spectrum (mull or solution) was identical with that

[29] Harper, *J.*, 1940, 1178.
[30] Griesebach and Ollis, *Experientia*, 1961, **17**, 4.

of the material, m. p. 233—235°; this may be an instance of polymorphism, common among rotenoids.

Nepseudin, crystallised from chloroform–methanol, had m. p. 115—116°, $[\alpha]_D^{20}$ 0° (in benzene or chloroform or 1 : 1 chloroform–methanol), $\lambda_{max.}$ 235 (4·74), 257i (4·08), 272 (3·94), and 335 (3·61) mμ, $\nu_{max.}$ 1686 (C=O), 1631, 1605, 1585, 1541, 1490 (aryl), $\nu_{max.}$ (mull) 1686i, 1680 (C=O), 1629, 1605, 1580, 1490 cm.$^{-1}$ [Found: C, 67·65; H, 4·95; OMe, 26·85. $C_{17}H_9O_3(OCH_3)_3$ requires C, 67·8; H, 5·05; 3OMe 26·3%]. The proton integral of the nuclear magnetic resonance spectrum shows 3OMe to a total of 18 protons.

Dolineone was isolated by Dr. P. J. Godin from *Pachyrrhizus erosus*. The ground beans were extracted with light petroleum and then with acetone. The acetone extracts were evaporated to a syrup which crystallised at 0°. The crystals were filtered off and washed with ether–light petroleum (1 : 2). Pale yellow crystals (14 g.) remained undissolved and these were extracted for 12 hr. (Soxhlet) with light petroleum (b. p. 60—80°) and then with chloroform. On storage, the chloroform extract deposited crude erosnin (2·1 g.) and then, on evaporation, a yellow solid (4 g.). The latter was chromatographed on alumina (A3) from benzene. Several colourless fractions showing a light blue fluorescence were obtained. After evaporation and crystallisation from acetone they yielded dolineone as needles, m. p. and mixed m. p. with the sample from *N. pseudopachyrrhiza* 235° (and spectral comparison).

Thin-layer chromatography on Kieselgel G (Merck) with benzene–chloroform (1 : 1) as eluent was useful for following the composition of mixtures. Some R_F values and colour reactions are:

	R_F	Fluorescein–Br_2	Iodine
1. Pachyrrhizone	0·39	Pale pink	Pale yellow
2. Dolineone	0·57	,,	Colourless
3. Erosnin	0·25	Orange-pink	Blue
4. Pachyrrhizin	0·35	Orange	Black
5. Neotenone	0·47	Deep pink	Yellow
6. Nepseudin	0·32	Pink	Orange

A mixture of 1—5 could be separated, although neotenone tended to form a broad spot and became partly involved in the dolineone spot. The technique was deceptive for tracing dolineone in natural extracts as an unidentified substance sometimes simulated its behaviour.

Dehydrogenation of Neotenone.—Neotenone (50 mg.) in acetone (15 ml.) was refluxed with active manganese dioxide (0·5 g.) for 3 hr. The manganese dioxide was filtered off and washed with chloroform, and the filtrate and washings were evaporated to small volume and diluted with methanol. Crystallisation from ethanol–chloroform gave *dehydroneotenone* (III), needles, m. p. 240—241°, $\lambda_{max.}$ 239 (4·61) and 308 (4·10) mμ. $\nu_{max.}$ 1658 (unsatd. ketone), 1629, 1592, 1549, 1506 (aryl) cm.$^{-1}$ (Found: C, 67·6; H, 3·85. $C_{19}H_{12}O_6$ requires C, 67·85; H, 3·60%).

Oxidation of Dehydroneotenone.—Dehydroneotenone (100 mg.) was added in portions to a mixture of ethanol (8 ml.), water (2 ml.), potassium hydroxide (0·5 g.) and 30% hydrogen peroxide (1 ml.). The mixture was stirred, then kept at 40° for 20 min. and more hydrogen peroxide (0·4 ml.) was added. After 10 min. more hydrogen peroxide (0·4 ml.) was added and then after another 5 min. had elapsed still more (0·4 ml.). The solution was kept at 45° (45 min.) and then heated to 70°. Cooling, acidification, and extraction with ether gave crystals, m. p. 100—125° (from ether–light petroleum). Two slow crystallisations from aqueous methanol gave 6-methoxypiperonylic acid, m. p. and mixed m. p. 147—149° with an authentic specimen (below). The mother-liquors from the ether–light petroleum crystallisation gave needles of 6-hydroxybenzofuran-5-carboxylic acid (VIII) (from methanol), m. p. and mixed m. p. 210° with an authentic specimen (below). The m. p. was depressed to 190° on admixture with authentic 4-hydroxybenzofuran-5-carboxylic acid (X), m. p. 225°, kindly supplied by Professor S. H. Harper.

Specimens of 6-hydroxybenzofuran-5-carboxylic acid were made as follows. 6-Hydroxycoumaranone was prepared by the Hoesch reaction between resorcinol and chloroacetonitrile followed by cyclisation of the resulting chloroacetoresorcinol.[12] The coumaranone was converted into the oxime and reduced with sodium amalgam in acetic acid–ethanol to the amine acetate.[31] Boiling with water gave 6-hydroxybenzofuran [31] which was carboxylated to give 6-hydroxybenzofuran-5-carboxylic acid, needles, m. p. 210° after vacuum-sublimation [lit.,[15] m. p. 210—215° (dependent on rate of heating)]. The acid was also made by Huang-Minlon

[31] Sonn and Patske, *Ber.*, 1925, **58**, 96.

reduction of 6-hydroxycoumaranone to 6-hydroxycoumaran [13] which was converted into 5-formyl-6-hydroxycoumaran with zinc cyanide–hydrogen chloride. The formyl compound had m. p. 107—108° (lit.,[13] m. p. 108°) and oxidation with alkaline silver oxide gave 6-hydroxycoumaran-5-carboxylic acid, m. p. 200° (from acetone–water) (lit.,[14] m. p. 198—201°). This coumaran acid could also be made by Kolbe carboxylation of 6-hydroxycoumaran. 6-Hydroxycoumaran-5-carboxylic acid (130 mg.) was converted into the *methyl ester* (95 mg.), m. p. 117—117·5° [from light petroleum (b. p. 40—60°)] (Found: C, 61·7; H, 5·4. $C_{10}H_{10}O_4$ requires C, 61·85; H, 5·2%). The ester (50 mg.), which gives a purple green colour with aqueous ferric chloride, was sublimed at 70°/0·05 mm. on to a glass-wool column (10 × 0·5 cm.) supporting 10% palladium–charcoal (150 mg.). The temperature was raised to 150° and the column was then washed with dilute aqueous potassium hydroxide and warmed on the steam-bath for 30 min. Acidification and ether-extraction gave a brown solid which when sublimed at 100—150°/0·05 mm. gave crystals (20 mg.); recrystallisation from aqueous methanol and sublimation then gave 6-hydroxycoumarone-5-carboxylic acid, m. p. 207—210°, identical with the material described above.

The authentic 6-methoxypiperonylic acid (VII) was made from 6-nitropiperonal.[32] This was reduced to 6-aminopiperonal, m. p. 105° (it is reported that yields are unsatisfactory unless commercial-grade ferrous sulphate is used: we find pure ferrous sulphate adulterated by adding a little ferric sulphate and ferric chloride makes a satisfactory reagent).[10] Diazotisation [10] gave 6-hydroxypiperonal, m. p. 125—126°, which was methylated to 6-methoxypiperonal, m. p. 111° (semicarbazone, m. p. 222°) (lit.,[10] m. p. 111—112°; semicarbazone, m. p. 222°). Oxidation of 6-methoxypiperonal with alkaline permanganate gave 6-methoxypiperonylic acid, m. p. 148° (lit.,[10,11] m. p. 147—147·5°, 148—149°).

Hydrolysis of Dehydroneotenone.—Dehydroneotenone (600 mg.) was refluxed in nitrogen for 3 hr. with 5% ethanolic potassium hydroxide (60 ml.). The solution was extracted with ether and on evaporation and crystallisation from methanol the deoxybenzoin, *6-hydroxy-5-(2-hydroxy-4,5-methylenedioxyphenylacetyl)benzofuran* (IV; R = R′ = H), m. p. 162—163°, $\nu_{max.}$ 1642 (chelated C=O) cm.$^{-1}$, was obtained (Found: C, 66·2; H, 4·45. $C_{18}H_{14}O_6$ requires C, 66·25; H, 4·3%). The deoxybenzoin gave a green colour with ethanolic ferric chloride and slowly dissolved in aqueous sodium hydroxide.

Conversion of the Deoxybenzoin (IV; R = R′ = H) *into Dehydroneotenone.*—The deoxybenzoin (25 mg.) was refluxed for 1 hr. with pyridine (0·5 ml.), piperidine (0·05 ml.), and ethyl orthoformate (0·5 ml.). Methanol (1 ml.) was added and, on cooling, dehydroneotenone (15 mg.), needles, m. p. and mixed m. p. 241°, was isolated.

Reduction of Neotenone with Potassium Borohydride.—Neotenone (100 mg.) in tetrahydrofuran (7 ml.) was treated with potassium borohydride (100 mg.) in 70% aqueous ethanol (5 ml.) containing potassium hydroxide (10 mg.). The mixture was kept at 20° for 24 hr., diluted with saturated aqueous ammonium chloride, and extracted with ether. Evaporation of the extracts gave a gum which crystallised from methanol to give the *alcohol* (V), fluffy needles, m. p. 215° (Found: C, 67·05; H, 4·9. $C_{19}H_{16}O_6$ requires C, 67·05; H, 4·75%).

Reduction of Dehydroneotenone with Potassium Borohydride.—Dehydroneotenone (100 mg.) in tetrahydrofuran (14 ml.) was treated at 60° for 90 min. with potassium borohydride (100 mg.) in 70% aqueous ethanol (10 ml.) containing potassium hydroxide (10 mg.). The mixture was kept at 20° for 30 min. and then diluted with saturated ammonium chloride solution and extracted with ether. The extracts were dried and evaporated; the residue crystallised from methanol to give the alcohol (V) (50 mg.), m. p. and mixed m. p. 215° with the specimen described above (and infrared comparison).

Oxidation of the Hydroxy-Compound (V).—The hydroxy-compound (40 mg.) in acetone (3 ml.) was added to chromic oxide (30 mg.) in acetone (2 ml.) and kept overnight. After filtration, the solution was poured into water and extracted with ether, to give (±)-neotenone (10 mg.), m. p. and mixed m. p. 179—180° (from methanol).

Dehydration of the Hydroxy-Compound (V).—The alcohol (110 mg.) in pyridine (1·5 ml.) was refluxed with phosphorus oxychloride (0·5 ml.) for 25 min. After cooling, the mixture was poured into water and extracted with ether. The extracts were washed, dried, and evaporated and the product crystallised from methanol to give *6-(2-methoxy-4,5-methylenedioxyphenyl)-7H-furo[3,2-g][1]benzopyran* (VI) as needles (40 mg.), m. p. 122°, $\lambda_{max.}$ 240 (4·35), 340 (4·27) mμ (Found: C, 70·5; H, 4·1. $C_{19}H_{14}O_5$ requires C, 70·8; H, 4·35%).

[32] Ekeley and Klemme, *J. Amer. Chem. Soc.*, 1925, **50**, 2711.

Oxidation of Dolineone.—Dolineone (40 mg.) in acetone (5 ml.) was shaken with active manganese dioxide (400 mg.) for 24 hr. After filtration the oxide was washed with hot chloroform, and the filtrate and washings were united and concentrated, to give 6a,12a-dehydrodolineone; m. p. 270° (decomp.) (from chloroform–methanol), $\nu_{max.}$ 1639 (α-unsatd. C=O), 1613, 1587, 1538, and 1499 (aryl) cm.$^{-1}$. The dehydro-compound (3 mg.) in glacial acetic acid (1·5 ml.) was kept at 20° with lead tetra-acetate (30 mg.) for 4 days. Chloroform (2 ml.) was added and the product was washed with water and sodium hydrogen carbonate solution, dried, and evaporated. The yellow gum was dissolved in chloroform (0·1 ml.); the infrared spectrum of the solution showed bands at 1748 (α-pyrone), 1730i, 1658 (unsatd. 12-ketone), and 1631 cm.$^{-1}$. Similar oxidation of dehydropachyrrhizone gave a gum with bands at 1754 (α-pyrone), 1656 (unsatd. 12-ketone), and 1623 cm.$^{-1}$.

Dehydropachyrrhizone (9 mg.), made by refluxing pachyrrhizone (15 mg.) in dioxan (10 ml.) with active manganese dioxide (150 mg.) for 3 hr., had m. p. 255—260° (decomp.) (lit.,[26] m. p. 259—261°). Pachyrrhizonone formed orange needles (from cyclopentanone), m. p. 349° (lit.,[26] m. p. 343°), and was made by treating pachyrrhizone with pentyl nitrite. It had $\nu_{max.}$ (mull) 1745 cm.$^{-1}$.

Reduction of Dolineone with Zinc Dust and Alkali.—Dolineone (10 mg.) and zinc dust (50 mg.) were refluxed for 2½ hr. with ethanol (1 ml.) and 10% aqueous potassium hydroxide (0·2 ml.). The product was filtered and the ice-cold filtrate was acidified and extracted with ether. The extracts were washed with aqueous potassium hydroxide, and the washings were shaken for 15 min. with dimethyl sulphate (0·05 ml.). Further dimethyl sulphate (0·05 ml.) was added, the whole shaken for 15 min., and the treatment repeated twice more. The solution was extracted with chloroform and these extracts were dried and evaporated to small volume. Thin-layer chromatography of a sample (Kieselgel G; elution with chloroform) showed several spots (iodine development), including one at R_F 0·65. The whole of the solution was then chromatographed as one line and the section of the plate corresponding to R_F 0·6—0·7 was scraped off and extracted with chloroform. The chloroform solution was concentrated and a portion was chromatographed as before alongside reference spots of the deoxybenzoin (IV; R′ = H, R = Me), and in admixture with it. All the spots had R_F 0·65 and were indistinguishable under treatment with ferric chloride (green-brown), iodine (yellow), and *p*-nitrobenzenediazonium chloride (yellow, becoming brown on treatment with aqueous sodium carbonate) as spray reagents. The degradation product and the authentic deoxybenzoin were also indistinguishable when benzene (R_F 0·3) and chloroform–ethanol (R_F 0·8) were used as developers. As a check on the method, a mixture of the deoxybenzoin (IV; R′ = H, R = Me) and monomethylpachyrrhol (IV; R′ = OMe, R = Me) was separable (double spot R_F 0·65 and 0·62) when chloroform was used as eluant.

The sample of pachyrrhol used was prepared by zinc and alkali reduction of pachyrrhizone and had m. p. 196—197° (lit.,[26] m. p. 194—197°); it gave a deep green ferric chloride colour. Methylation with dimethyl sulphate and aqueous potassium hydroxide gave monomethylpachyrrhol, m. p. 171° (lit.,[26] m. p. 173—174°).

Dehydronepseudin.—Nepseudin (300 mg.) in dioxan (10 ml.) was refluxed with active manganese dioxide (3 g.) for 3 hr. After the solid had been removed and washed with hot chloroform, the filtrates were evaporated and the residue was crystallised from methanol, to give *dehydronepseudin* (XIX) as needles (42 mg.), m. p. 158—160°, $\lambda_{max.}$ 245 (4·64) and 325 (3·92) mμ, $\nu_{max.}$ (mull) 1647 (α-unsaturated C=O), 1621, 1603, 1580, 1541, 1493 (aryl) cm.$^{-1}$ (Found: C, 68·2; H, 4·8. $C_{20}H_{16}O_6$ requires C, 68·2; H, 4·55%). A by-product (2 mg.), m. p. 241·5—242°, was also observed.

Hydrolysis of Dehydronepseudin.—The dehydro-compound (60 mg.) was refluxed with 5% ethanolic potassium hydroxide (1 ml.) for 3 hr. The solution was poured into water, acidified, and extracted with chloroform. The extracts were washed with sodium hydrogen carbonate solution, dried, and evaporated, and the residue was crystallised from methanol, to give the *6-hydroxy-5-(2,3,4-trimethoxyphenylacetyl)benzofuran* (XX) (30 mg.) as pale yellow plates, m. p. 118—119°, $\lambda_{max.}$ 235 (4·66), 258 (4·00), 270i (3·86), and 345 (3·60), $\nu_{max.}$ (mull) 1686w, 1645m, 1629m, 1595, 1536, and 1488 cm.$^{-1}$ (Found: C, 66·65; H, 5·25. $C_{19}H_{18}O_6$ requires C, 66·65; H, 5·25%).

Oxidation of Dehydronepseudin with Hydrogen Peroxide.—Dehydronepseudin (100 mg.) was added slowly to potassium hydroxide (500 mg.) in ethanol (8 ml.) and water (2 ml.) containing 30% hydrogen peroxide (1 ml.). The suspension was kept at 40° for 65 min., 3 further portions

of hydrogen peroxide (0·4 ml. each) being added at approx. 5 min. intervals. After the solution had been heated to 70°, cooled, filtered, and acidified, the mixed acids were extracted with chloroform and ether. Evaporation gave a gum (47 mg.) which solidified on trituration with light petroleum. A portion (40 mg.) was crystallised from aqueous methanol, and the crystals were sublimed at 140°/0·1 mm. to give 6-hydroxybenzofuran-5-carboxylic acid (5 mg.), m. p. and mixed m. p. 211° (and infrared comparison). Admixture with 4-hydroxybenzofuran-5-carboxylic acid, m. p. 225°, depressed the m. p. to 195°.

The liquors from the crystallisation were concentrated and, on cooling, a little solid was deposited and was filtered off. The filtrate was extracted with ether, and the ethereal extracts gave, on evaporation, a gum which crystallised (m. p. 96°, 10 mg.), on trituration with light petroleum. Recrystallisation from ether–light petroleum gave 2,3,4-trimethoxybenzoic acid m. p. and mixed m. p. 99° (and infrared comparison). It gave no colour with ferric chloride. Authentic material was prepared from pyrogallol-4-carboxylic acid, m. p. 219° (decomp.).[33] The acid was treated with diazomethane and then methylated with methyl iodide and potassium hydroxide until it gave no ferric chloride colour. The oily ester was hydrolysed; the acid, crystallised from ether–light petroleum, had m. p. 99° (lit.,[22] m. p. 99°).

We thank Dr. J. W. Lown for the nuclear magnetic resonance information. The investigation was aided by financial support from Shell Research Limited and the University of London Research Fund. A grant from the Royal Society for the purchase of counter-current equipment is gratefully acknowledged. We also express our gratitude to Mr. A. G. Kenyon of the Tropical Products Institute for his efforts in obtaining plant materials.

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Strand, London, W.C.2. [*Received, August 1st*, 1962.]

[33] Kostanecki, *Ber.*, 1885, **18**, 3205.