Tetrahedron. Vol. 26, pp. 3023 to 3027. Pergamon Press 1970. Printed in Great Britain

CHEMICAL STUDY OF INDIAN YAM BEANS (*PACHYRRHIZUS EROSUS*)

ISOLATION OF TWO NEW ROTENOIDS: 12a-HYDROXYDOLINEONE AND 12a-HYDROXYPACHYRRHIZONE

M. KRISHNAMURTI, Y. R. SAMBHY and T. R. SESHADRI
Department of Chemistry, University of Delhi, Delhi-7, India

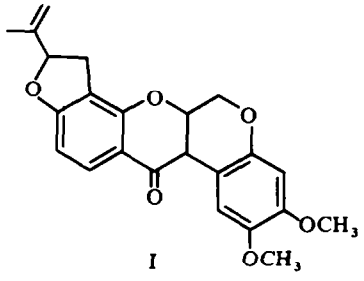
(*Received UK* 20 *December* 1969; *Accepted for publication* 11 *March* 1970)

Abstract—Our study indicates considerable variation in the chemical components of the beans depending on habitat and season. Besides the eight compounds obtained earlier two new rotenoids have now been isolated and their constitutions established as 12a-hydroxydolineone and 12a-hydroxypachyrrhizone.

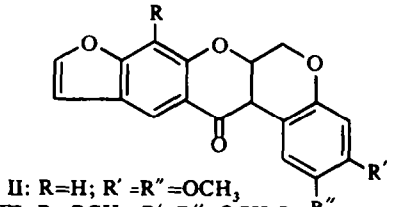
Pachyrrhizus erosus (Sankhalu) is cultivated throughout India and particularly in West Bengal. The seeds which are rich in oil are toxic and the toxicity is comparable to that of the rotenone bearing roots of Derris and Lonchocarpus. The earliest study was made by Nag *et al*[1] who considered the toxic principle to be a saponin. They also determined the acid composition of the seed fat but a more detailed examination was made by Broadbent and Shone[2] and by Pathak and Agarwal.[3] The variation in the results of the above workers may be seasonal or geographical or both.

Earlier work[4-8] showed the presence of eight closely related compounds: an isoflavone, dehydroneotenone (V), an isoflavanone, neotenone (VI), four rotenoids, rotenone (I), erosone (II), pachyrrhizone (III) and dolineone (IV) and two furano-3-phenyl coumarins, pachyrrhizin (VII) and erosnin (VIII). The isolation of these from the same plant is of considerable biogenetic interest and this has been discussed by Krishnamurti and Seshadri.[9]

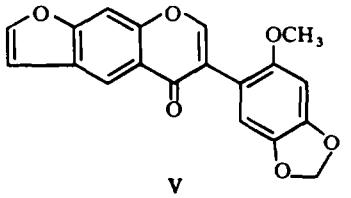
In an earlier publication[10] a synthesis of isoelliptic acid (IX), the intermediate acid required for the synthesis of erosone (II), was reported. It was considered desirable to make a direct comparison of the acid with an authentic sample in view of the slight difference in mp. As a sample of the acid was not obtainable before an investigation of *Pachyrrhizus erosus* available in India was taken up, it was done twice; first with 500 g of the seeds collected in 1966 and later with 2·5 Kg collected in 1968. The earlier sample did not yield erosone but gave pachyrrhizin (VII), erosnin (VIII), dehydroneotenone (V) and dolineone (IV). The subsequent larger sample gave better results. The defatted seeds were extracted with ether. The ether extract was separated into its components by chromatography over neutral alumina. The earlier fractions gave more of the oil followed by erosnin, neotenone, dolineone, erosone, pachyrrhizin, rotenone, dehydroneotenone and pachyrrhizone. It may be mentioned here that the yields of these compounds varied from traces (20 mg, erosnin and erosone) to as high as 3·5 g (pachyrrhizin). The yields of the other compounds are: dolineone (205 mg), neotenone (210 mg), dehydroneotenone (300 mg), rotenone (1 g) and pachyrrhizone (1·75 g). Degradation of erosone to isoelliptic acid could not be done because of the small quantity of the compound isolated. When the column was subsequently eluted with



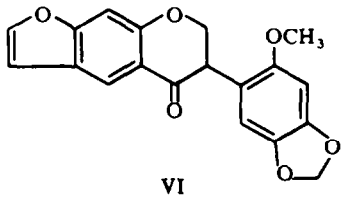
I



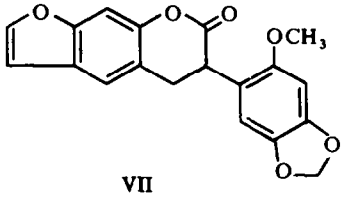
II: R=H; R′ =R″=OCH_3
III: R=OCH_3; R′ R″=OCH_2O
IV: R=H; R′ R″=OCH_2O



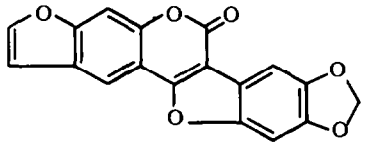
V



VI



VII

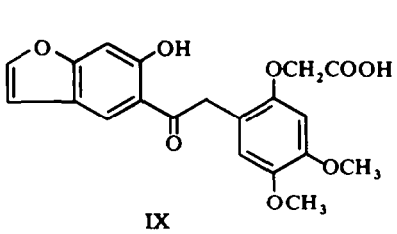


VIII

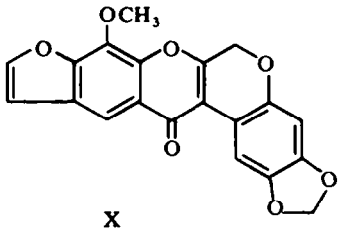
chloroform and then with chloroform-acetone (3 : 1) brownish resinous products were obtained. By further chromatography of the benzene solution of these products on silica gel using light petroleum with increasing amounts of benzene as eluent, three compounds were isolated and these gave positive colour tests for rotenoids. The first compound was identified as dehydropachyrrhizone (X) by comparison with an authentic sample (mixed m.p. and IR spectrum). The second compound (145 mg), m.p. 180–181°, had $[\alpha]_D$ $CHCl_3$ + 137°. Its UV and IR spectra indicated it to be closely related to dolineone. The analytical values and its mol wt (352 from mass spectrum) agreed with the formula $C_{19}H_{12}O_7$ indicating an extra OH group confirmed by the preparation of an acetate. The OH was not phenolic and hence the constitution of 12a-hydroxydolineone (XI) was proposed for this compound and this was confirmed by dehydration of the compound to dehydrodolineone which agreed with a synthetic sample in all respects. Further confirmation was obtained by the transformation of dolineone to *cis* (±)-12a-hydroxydolineone which agreed completely with the natural product in UV (in EtOH) and IR (in $CHCl_3$).

The third compound (110 mg), m.p. 214°, had $[\alpha]_D CHCl_3$ + 126°. The close similarity of its UV and IR spectra with those of pachyrrhizone and its molecular formula $C_{20}H_{14}O_8$ indicated that the compound may be 12a-hydroxypachyrrhizone (XII). This was supported by its properties and the formation of an acetate. Confirmation was provided by dehydration and comparison of the product with authentic

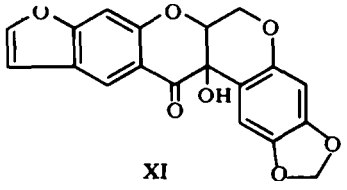
dehydropachyrrhizone; further *cis*(±)-12a-hydroxypachyrrhizone prepared from pachyrrhizone by aerial oxidation in alkaline solution agreed with the natural sample in spectral characteristics.



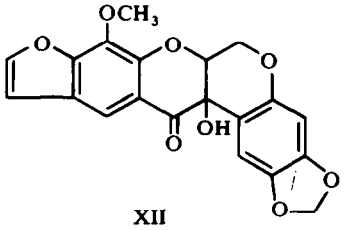
IX



X



XI



XII

The present isolation of 12a-hydroxyrotenoids from *Pachyrrhizus erosus* and of (-)-milletosin and (−)-tephrosin from *Milletia dura* by Ollis *et al*[11] would suggest that these 12a-hydroxyrotenoids are also natural products and not artefacts as considered before. They may arise by a process of phytochemical oxidation at the reactive 12a-position. Since they can undergo easy reduction they and the corresponding rotenoids probably constitute oxidation-reduction systems. Further the isolation of dehydropachyrrhizone may suggest that the dehydrorotenoids are also natural products arising from dehydrogenation.

EXPERIMENTAL

NMR spectra were determined in $CDCl_3$ using a Varian A-60 spectrometer and TMS as internal standard, m.ps using a Kofler hot stage microscope are not corrected.

Extraction: The ground seeds (2·5 kg) were successively extracted with the following solvents. Extraction (70 hr) with light petroleum (b.p. 60–80°) in a soxhlet type apparatus yielded a bright yellow oil (880 g, 35%). A subsequent extraction (70 hr) with ether in the same extracter yielded fraction E_1 (43·5 g) and a further continuous extraction (50 hr) with ether yielded fraction E_2 (6·2 g).

Examination of fraction E_1. Fraction E_1 was refluxed for 45 min with light petroleum (b.p. 60–80°; 2 × 200 ml portions) to remove fatty material. The remaining solid (33·2 g) was dissolved in 250 ml dry benzene and chromatographed over alumina (neutral, 1 kg) using successively benzene, benzene with 10,

25 and 50% $CHCl_3$, $CHCl_3$, $CHCl_3$ with 10, 20 and 40% acetone as eluting solvents and collecting 400 ml fractions. They were studied on TLC, similar fractions were combined and the mixture separated by rechromatography and fractional crystallization. Identification of the compounds was effected by m.p., TLC and spectral data in comparison with authentic samples wherever possible.

Benzene elution gave the following: fractions 1-4 erosnin, 5-21 neotenone, dolineone and erosone, 22–40 dolineone and pachyrrhizin, 41–50 pachyrrhizin and rotenone. Benzene–$CHCl_3$ (10%) yielded pachyrrhizin and rotenone. Pachyrrhizin, rotenone and dehydroneotenone were isolated from benzene–$CHCl_3$ (25%). The residue obtained from benzene–$CHCl_3$ (50%) was combined with fraction E_2 and was separated into dehydroneotenone and pachyrrhizone.

The $CHCl_3$ and $CHCl_3$–acetone fractions yielded 2·15 g of a brownish resin which gave positive Rogers–Calamari test. The resin was taken up in benzene (10 ml) and chromatographed on silica gel (75 g) using light petroleum with increasing amounts of benzene as eluent. Three compounds were isolated; dehydropachyrrhizone (80 mg), 12a-hydroxydolineone (145 mg) and 12a-hydroxypachyrrhizone (110 mg).

Dehydropachyrrhizone (X). The compound separated from $CHCl_3$–MeOH as pale yellow needles, m.p. 260–262°(d). It gave a blue colour in the Rogers–Calamari test, a negative Durham test and a positive test for methylenedioxy group. The UV and IR spectra of the compound and of authentic dehydropachyrrhizone were identical; λ_{max}^{dioxan} 241 (4·5), 275 (4·4), 311 (4·25) mμ; ν_{max}^{KBr} 1639, 1613, 1587, 1500, 1031 and 935 cm^{-1}.

(+)-12a-*Hydroxydolinenone* (XI). Crystallization from $CHCl_3$–MeOH yielded the compound as colourless needles m.p. 180–181°. It gave positive Rogers–Calamari test and Labat test but negative Durham test. It gave no colour with ethanolic $FeCl_3$ and was insoluble in NaOH aq. (Found: M^+ 352; C, 65·2; H, 3·6; $C_{19}H_{12}O_7$ requires: M, 352; C, 64·8; H, 3·4%); λ_{max}^{EtOH} 237 (4·54), 276 (3·84), 304 (3·66), 338 infl (3·64) mμ; ν_{max}^{nujol} 3571, 1685, 1635, 1504, 1041, 935, 877 cm^{-1}; NMR spectrum (in $CDCl_3$) δ 8·25, (11-H); δ 6·75, (J=3 c/s) and δ 7·58, (J=3 c/s) (H on furan ring); δ 7·05, (8-H); δ 6·52, and δ 6·58, (1-H, 4-H); δ 5·84, (—O—CH_2—O—); δ 4·6 mt (6-H, 6a-H, 12a-OH). The IR spectrum (in $CHCl_3$) was identical with that of a synthetic sample (see below).

(+)-12a-*Hydroxypachyrrhizone* (XII). The compound separated from $CHCl_3$-MeOH as colourless needles, m.p. 214°. It gave a blue colour in the Rogers–Calamari test, positive Labat test and a negative Durham test. It was insoluble in NaOH aq and gave no colour with ethanolic $FeCl_3$. (Found: M⁺382; C, 62·9, H, 4·1; $C_{20}H_{14}O_8$ requires: M,382; C, 62·8; H, 3·7%); λ_{max}^{EtOH} 244 (4·5), 284 (3·8), 341 (3·45) mμ; ν_{max}^{nujol} 3571, 1681, 1623, 1500, 1035, 935, 870 cm^{-1}. NMR spectrum (in $CDCl_3$) δ 7·85, (11-H); δ 6·7, (J = 3 c/s), δ 7·55, (J = 3 c/s) (H on furan ring); δ 6·45, δ 6·5 (1-H, 4-H); δ 5·8, (—O—CH_2—O—); δ 4·6, (6-H, 6a-H, 12a-OH); δ 4·1, (—OCH_3). The IR spectrum (in $CHCl_3$) was identical with that of a synthetic sample.

Oxidation of dolineone to (±)-12a-*hydroxydolineone*. 0·5N NaOH (0·3 ml) was added to a soln of dolineone (25 mg) in dioxan–MeOH (1 : 2, 15 ml) and the mixture aerated at room temp for 16 hr. The soln was concentrated under reduced press and diluted with water. The ppt was filtered off and fractionally crystallized from $CHCl_3$–MeOH to yield two compounds; a less soluble and minor (±) *trans*-isomer and a more soluble and major (±) *cis*-isomer. The latter on further crystallization yielded *cis* (±) 12a-hydroxydolineone as colourless cubes, m.p. 192–194°; its UV (in EtOH) and IR (in $CHCl_3$) were identical with the natural sample.

Oxidation of pachyrrhizone to (±) 12a-*hydroxypachyrrhizone*. 0·5N NaOH (0·5 ml) was added to a soln of pachyrrhizone (40 mg) in dioxan-MeOH (2 : 1, 20 ml) and the reaction carried out as above; *cis* (±)-12a-hydroxypachyrrhizone separated from $CHCl_3$-MeOH as colourless cubes, m.p. 232–234°; its UV (in EtOH) and IR (in $CHCl_3$) were identical with the natural sample.

Acetylation of 12a-hydroxydolineone and 12a-*hydroxypachyrrhizone*. 12a-Hydroxydolineone (60 mg) in dry pyridine (0·5 ml) was treated with Ac_2O (0·25 ml) and the mixture was left overnight at room temp and worked up as usual. The acetate separated from $CHCl_3$–MeOH as colourless needles (62 mg), m.p. 242° (pre-heated block at 239°). NMR spectrum (in $CDCl_3$) δ 2·20, (—CO—CH_3); δ 4·38, and δ 4·72, (J = 12 c/s and 14 c/s —O—CH—CH_2—O—); δ 5·58 unresolved (—O—CH—CH_2—O—); δ 5·95, (—O—CH_2—O—); δ 6·6, (4-H); δ 7·0, (1-H); δ 6·84, and 7·66, (H on furan ring); δ 7·15, (8-H); δ 8·4, (11-H).

12a-Hydroxypachyrrhizone (50 mg) on acetylation under similar conditions gave the acetate (50 mg), m.p. 232–233° (preheated block at 230°). NMR spectrum (in $CDCl_3$) δ 2·15, (—CO—CH_3); δ 4·12, (—OCH_3); δ 4·34, and δ 4·74, (J = 12 c/s and 14 c/s —O—CH—CH_2—O—); δ 5·52 unresolved

(—O—CH—CH_2—O—); δ 5·90, (—O—CH_2—O—); δ 6·51, (4-H); δ 6·92, (1-H); δ 6·8, and 7.62, (H on furan ring); δ 8·0, (11-H).

Dehydrodolineone. Acetate of 12a-hydroxydolineone (10 mg) was refluxed with Ac_2O (0·5 ml), NaOAc (20 mg) and a drop of AcOH for 20 min. After cooling, MeOH (2 ml) was added and the mixture left overnight. The dehydrodolineone which separated as a solid was purified by chromatography (silica gel) using benzene as eluent. It separated from $CHCl_3$—MeOH as pale yellow needles (5 mg), m.p. 275–277° (dec) undepressed by admixture with an authentic sample prepared from dolineone by oxidation with active MnO_2 in acetone soln.[8] The UV and IR spectra of the samples were identical λ_{max}^{dioxan} 237 (4·37), 275 (4·15), 310 (4·11) mμ; ν_{max}^{KBr} 1639, 1613, 1587, 1538, 1040 and 935 cm^{-1}.

12a-Acetoxydolineone (15 mg) could also be converted into dehydrodolineone by heating it in an oil-bath (245°) under reduced press. The acetate first melts and solidifies immediately. Crystallization of the residue from $CHCl_3$—MeOH gave dehydrodolineone as pale yellow needles (10 mg), m.p. and mixed m.p. with the above specimen 275–277° (dec).

Dehydropachyrrhizone. 12a-Acetoxypachyrrhizone (10 mg) was refluxed with Ac_2O (0·5 ml), NaOAc (20 mg) and a drop of AcOH for 15 min and the product isolated as above. Dehydropachyrrhizone crystallized from $CHCl_3$—MeOH as yellow cubes, m.p. 257–259° (dec) undepressed by admixture with an authentic sample prepared from pachyrrhizone.[8] The UV and IR spectra of synthetic and natural samples were identical; λ_{max}^{dioxan} 241 (4·6), 275 (4·4), 311 (4·27) mμ; ν_{max}^{KBr} 1639, 1613, 1587, 1500, 1031 and 935 cm^{-1}.

12a-Acetoxypachyrrhizone (15 mg) when heated in an oil bath as described above yielded dehydropachyrrhizone (12 mg) m.p. and mixed m.p. with the above specimen 257–259° (dec).

REFERENCES

[1] N. C. Nag, H. N. Banerjee and A. K. Pain, *Trans. Bose Research Inst.* **11**, 83 (1935–1936)

[2] J. H. Broadbent and G. J. Shone, *J. Sci. Food Agric.* **14**, 524 (1963)

[3] S. P. Pathak and B. C. L. Agarwal, *Indian J. Appl. Chem.* **31**, 101 (1968)

[4] L. B. Norton and R. Hansberry, *J. Am. Chem. Soc.* **67**, 1609 (1945)

[5] H. Bickel and H. Schmid, *Helv. Chim. Acta* **36**, 664 (1953)

[6] E. Simonitsch, H. Frei and H. Schmid, *Monatsh.* **88**, 541 (1957); P. Rajagopalan and T. I. Kosak, *Tetrahedron Letters* **No 21**, 5 (1959)

[7] J. Eisenbeiss and H. Schmid, *Helv. Chim. Acta* **42**, 61 (1959); J. N. Chatterjea and N. Prasad, *Chem. Ber.* **97**, 1252 (1964)

[8] L. Crombie and D. A. Whiting, *J. Chem. Soc.* 1569 (1963)

[9] M. Krishnamurti and T. R. Seshadri, *Curr. Sci.* **35**, 167 (1966)

[10] V. Chandrashekar, M. Krishnamurti and T. R. Seshadri, *Ibid.* **36**, 623 (1967)

[11] W. D. Ollis, C. A. Rhodes and I. O. Sutherland, *Tetrahedron* **23**, 4741 (1967)