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THE EXTRACTIVES OF *MILLETTIA DURA* (DUNN)

THE CONSTITUTIONS OF DURLETTONE, DURMILLONE, MILLDURONE, MILLETTONE AND MILLETTOSIN

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Abstract The seeds of *Millettia dura* (Dunn) have yielded durlettone, durmillone, milldurone, (−)-millettone, (−)-millettosin, (−)-rotenone, (−)-tephrosin, (±)-tephrosin, and 6$_a$,12$_a$-dehydrodeguelin. The consitutions of the new isoflavones durlettone (I), durmillone (IV), and milldurone (VIII) have been established.

Millettone is shown to be the new rotenoid (XIX). The natural co-occurrence of the rotenoids, (−)-millettone (XIX) and (−)-rotenone (XX), with the 12$_a$-hydroxyrotenoids, (−)-millettosin (XXI) and (−)-tephrosin (XXII), and the 6$_a$,12$_a$-dehydrorotenoid, 6$_a$,12$_a$-dehydrodeguelin (XII), is established. The relation of these observations to the question of the natural occurrence of 12$_a$-hydroxyrotenoids and 6$_a$,12$_a$-dehydrorotenoids is discussed

ALTHOUGH the number of *Millettia* species is large,[1] their phytochemical examination has been limited to only a few cases.[2] The position of the *Millettia* genus in the *Leguminosae* family suggests that these plants could be sources of isoflavonoids[3] and rotenoids[4] and this is compatible with various claims that some *Millettia* species show insecticidal and piscicidal activities.[2] These activities have often been attributed to rotenone but the evidence that the active constituent is in fact rotenone is in most cases rather slight. Preliminary reports on the extractives of various *Millettias* are available including *M. taiwaniana*,[5] *M. pachycarpa*,[6 13] *M. reticulata*,[14] *M. pulchra*,[14]

[1] S. T. Dunn, *J. Botany* **49**, 211 (1911); *J. Linn. Soc. Bot.* **41**, 223 (1912)

[2] H. J. Holman, *A Survey of Insecticidal Materials of Vegetable Origin*, Imperial Institute, London (1940); R. E. Heal, E. F. Rogers, R. T. Wallace and O. Starnes, *Lloydia* **13**, 89 (1950); L. Feinstein and M. Jacobson, *Fortsch. Chem. org. Nat.* **10**, 424 (1953); R. G. W. Spickett, *Colonial Plant and Animal Products* **5**, 288 (1955)

[3] K. Venkataraman, *Fortsch. Chem. org. Nat.* **17**, 1 (1959); W. D. Ollis, *The Chemistry of Flavonoid Compounds* (Edited by T. A. Geissman) p. 353. Pergamon, Oxford (1961).

[4] [a] W. D. Ollis, *Proceedings of the Symposium on Phytochemistry* p. 128. Hong Kong University Press (1961); [b] I. Crombie, *Fortsch. Chem. org. Nat.* **21**, 275 (1963).

[5] T. Kariyone, K. Atsumi and M. Shimada, *J. Pharm. Soc. Japan* No. 500, 739 (1923); *Chem. Abstr.* **18**, 408 (1924)

[6] Chin-Pi Chen, *Science China* **19**, 1405 (1935); *Chem. Abstr.* **30**, 226 (1936)

[7] T. Ghose and S. Krishna, *Curr. Sci.* **6**, 57 (1937); *Chem. Abstr.* **32**, 5574 (1938).

[8] Shin-Foon Chin, Sping Lin and Yee Som Chiu, *J. Econ. Entom.* **35**, 80 (1942); *Chem. Abstr.* **36**, 5584 (1942).

[9] C. S. Lee and R. Hansberry, *J. Econ. Entom.* **36**, 915 (1943); *Chem. Abstr.* **38**, 3081 (1944).

[10] Shin-Foon Chin, Sping Lin and Ching-Yung Hu, *Canton Univ. Coll. Agr. Pub.* 1 (1945); *Chem. Abstr.* **41**, 2201 (1947).

[11] F. Tattersfield, C. Potter, K. A. Lord, E. M. Gilham, M. J. Way and R. J. Stoker, *Kew Bulletin* No. 3, 343 (1948)

[12] Shin-Foon Chin, *J. Sci. Food Agric.* **1**, 437 (1950).

[13] T. D. Mukerjea, *J. Sci. Ind. Res. India* **14C**, 169 (1955); T. D. Mukerjea and R. L. Tripathi, *Ibid.* **15C**, 106 (1956)

[14] Biennial Report of the National Agricultural Research Bureau. *Rev. Appl. Entom.* **27A**, 449 (1938); *Chem. Abstr.* **34**, 4512 (1940)

M. laurentii,[11] *M. versicolor*,[11] and *M. manii*,[11] but the only detailed study is that due to Clark.[15] He examined the seeds of *M. ferruginea* (Hochst.)* and identified rotenone and reported the isolation of $6_a,12_a$-dehydrorotenone, tephrosin, and two unknown compounds, m.p. 189–190° and m.p. 164–165°, formulated as $C_{19}H_{14}O_4(OMe)_2$ and $C_{22}H_{17}O_5(OMe)$ respectively. As Clark's isolation[15] of the latter four compounds involved exposure of the extract to alkali, their description as "natural products" has always been suspect.[4b,16-18] We decided to re-examine this matter and now report our study of the extractives of the seeds of *M. dura* (Dunn).*

The isolation of ten aromatic type natural products is described including three isoflavones, six members of the rotenoid class, and a compound of the chalkone type. The elucidation of the constitutions of nine of these compounds is now discussed.

The isoflavones isolated from M. dura

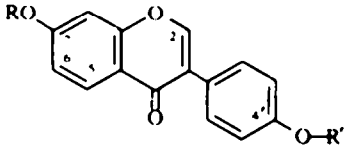
Durlettone. This compound, $C_{20}H_{17}O_3(OMe)$, showed UV [λ_{max} 260 mμ (ε 26,400), 297 mμ (ε 9990)] and IR [ν_{CO} 1640 cm^{-1}] spectral characteristics suggesting an isoflavone structure.[3,19] This was clearly supported by its NMR spectrum which showed signals assignable to the isoflavonoid 2-H (τ 2·00, singlet), one methoxyl group (τ 6·05, singlet), and a γγ-dimethylallyl group[20-23] (τ 4·43, triplet, H_1; τ 5·39, doublet, H_2; τ 8·19, broad singlet, H_6). The doublet signal of the methylene group of the dimethylallyl residue is at considerably lower field (τ 5·39) than the chemical shift normally observable for the methylene group (~τ 6·7) of a C-γγ-dimethylallyl substituent on an aromatic ring. This required the presence of an O-γγ-dimethylallyl group[24] so that durlettone could now be given the partial structure $C_{15}H_8O_2(OMe)$-(O CH_2—CH=CMe_2). Signals due to seven aromatic protons were discernible and these could be readily analysed in terms of an A_2X_2 system for a *p*-disubstituted aromatic ring [τ_A 2·41, τ_X 2·94 (J_{AX} = 9 c/s)] and the ABX system characteristic of a 1,2,4-trisubstituted benzene ring [τ_X 1·70, τ_A 2·93, τ_B 3·07 (J'_{AX} = 9 c/s, J'_{AB} = 2 c/s)]. It was also clear from the low field doublet of the ABX system [τ 1·70 (J = 9 c/s)] that this signal could be assigned to a proton at position 5 of an isoflavone skeleton, and this leads to two possible structures for durlettone (I and II) both of which have oxygen substituents at positions 7 and 4',

* *M. dura* (Dunn) is synonymous with *M. ferruginea* (Harms.) but the former name is considered to have taxonomic precedence. Clark examined *M. ferruginea* (Hochst.) and its further examination by R. J. Highet and P. F. Highet is to be reported in a forthcoming paper (*J. Org. Chem.*, in the press). *M. ferruginea* (Harms.) is not equivalent to *M. ferruginea* (Hochst.).

We thank Drs. R. J. Highet and P. F. Highet for information regarding their studies prior to publication.

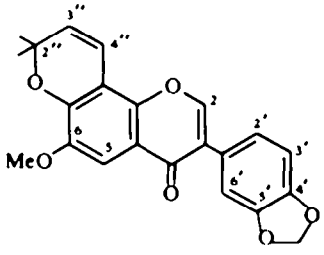
[15] E. P. Clark, *J. Am. Chem. Soc.* **65**, 27 (1943).
[16] H. Grisebach and W. D. Ollis, *Experientia* **17**, 4 (1961).
[17] L. Crombie and P. J. Godin, *J. Chem. Soc.* 2861 (1961).
[18] F. M. Dean, *Naturally Occurring Oxygen Ring Compounds* p. 501. Butterworths, London (1963).
[19] L. Jurd, *The Chemistry of Flavonoid Compounds* (Edited by T. A. Geissman) p. 107. Pergamon, Oxford (1961).
[20] B. F. Burrows, W. D. Ollis and L. M. Jackman, *Proc. Chem. Soc.* 177 (1960).
[21] W. D. Ollis, M. V. J. Ramsay, I. O. Sutherland and S. Mongkolsuk, *Tetrahedron* **21**, 1453 (1965).
[22] R. B. Bates and D. M. Gale, *J. Am. Chem. Soc.* **82**, 5749 (1960).
[23] R. B. Bates, R. H. Carnighan, R. O. Rakutis and J. H. Schauble, *Chem. & Ind.* **40**, 1020 (1962).
[24] W. D. Ollis and I. O. Sutherland, *Recent Developments in the Chemistry of Natural Phenolic Compounds* (Edited by W. D. Ollis) p. 74. Pergamon, London (1961).

positions which are most frequently oxygenated in natural isoflavones.[3] The presence of an O-γγ-dimethylallyl group in durlettone was fully confirmed by acidic hydrolysis which gave 4'-hydroxy-7-methoxyisoflavone (III). This established the constitution (I) for durlettone which was confirmed by synthesis. O-Alkylation of 4'-hydroxy-7-methoxyisoflavone (III) with γγ-dimethylallyl bromide gave durlettone (I). The isomer II was also synthesized from formononetin: it was clearly different from the natural product.

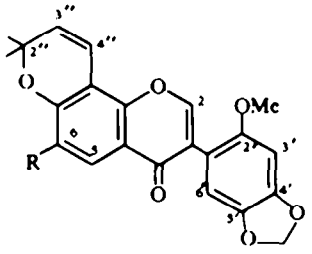


I: R = Me; R' = CH_2 $CH{=}CMe_2$ Durlettone
II: R = CH_2 $CH{=}CMe_2$; R' = Me
III: R = Me; R' = H

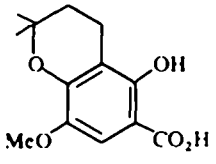
Durmillone. The NMR spectrum of durmillone, $C_{21}H_{15}O_5(OMe)$, led directly to consideration of the structure IV and this opinion was immediately reinforced by reference to the NMR spectra of jamaicin[25, 26] (V) and ichthynone[26] (VI) (Table 1). These spectra showed a striking correspondence in the chemical shifts of corresponding protons with the exception that durmillone showed an ABX system characteristic of 3',4'-methylenedioxyisoflavones.[27] Further support for the structure IV for dur-



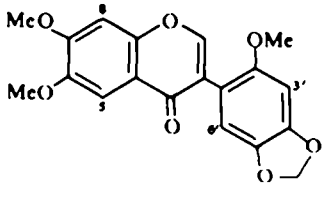
IV: Durmillone



V: R = H Jamaicin
VI: R = OMe Ichthynone



VII



VIII: Milldurone

[25] J. A. Moore and S. Eng, *J. Am. Chem. Soc.* **78**, 395 (1956); A. L. Kapoor, A. Aebi and J. Büchi, *Helv. Chim. Acta* **40**, 1574 (1957); O. A. Stamm, H. Schmid and J. Büchi, *Ibid.* **41**, 2006 (1958)
[26] J. S. P. Schwarz, A. I. Cohen, W. D. Ollis, E. A. Kaczka and L. M. Jackman, *Tetrahedron* **20**, 1317 (1964).
[27] W. D. Ollis and R. E. Wheeler, forthcoming publication.

TABLE 1. CHEMICAL SHIFTS (τ) FOR THE INDICATED PROTONS IN THE NMR SPECTRA OF DURMILLONE, JAMAICIN, ICHTHYNONE, AND MILLDURONE

Location of protons or substitutents	2-H	5-H	6-H	8-H	2'-H	3'-H	6'-H	2''-*gem*-Me_2	3''-H	4''-H	3', 4' CH_2O_2	2'-OMe	6-OMe	7-OMe
Durmillone (IV)	2·08	2·43			3·02*	3·14*	2·89*	8·45	4·27 (d, J = 10)	3·19 (d, J = 10)	4·02		6·04	
Jamaicin[26] (V)	2·08	1·97 (d, J = 8·5)	3·15 (d, J = 8·5)			3·38	3·15	8·51	4·28 (d, J = 10)	3·20 (d, J = 10)	4·05	6·28		
Ichthynone[26] (VI)	2·11	2·47				3·39	3·20	8·44	4·29 (d, J = 10)	3·21 (d, J = 10)	4·07	6·28	6·06	
Milldurone (VIII)	2·10	2·37		3·12		3·37	3·17				4·05	6·27	6·02	6·02

* ABX system (H_A τ 3·14, H_B τ 3·02, H_X τ 2·89; J_{AB} = 8·0 c.s, J_{BX} = 1·6 c.s, J_{AX} = 0·6 c.s). Note that these protons are numbered arbitrarily in formula (IV) to indicate their corresponding relation to formulae (V and VI).

Proton counts. All signals have the appropriate integrated intensities.

Multiplicity of signals. Unless otherwise indicated, all signals are singlets. For other cases d = doublet and the coupling constant J is given in c.s.

millone was provided by a comparison of its UV spectrum with the spectra of similar isoflavones[28] (Table 2). The UV spectrum of durmillone is very similar to that of jamaicin and ichthynone. In particular it may be noted that they all exhibit high intensity absorption in the 320–340 mμ region,[28] yet this is usually not regarded as being typical of compounds of the isoflavone type.[3, 19]

Catalytic hydrogenation of durmillone (IV) reduced the chromene double bond giving 3″,4″-dihydrodurmillone which on degradation with alkaline hydrogen peroxide gave the salicylic acid (VII) and piperonylic acid. This established the constitution IV proposed for durmillone since we had already rigorously established the structure for the salicylic acid (VII) during our investigation of the structure of ichthynone (VI).[26, 28]

Milldurone. The UV spectrum of milldurone, $C_{19}H_{16}O_7$, was very similar to that of ichthynone (Table 2) and suggested that they both had a similar oxygenation pattern. Its NMR spectrum showed the presence of one methylenedioxy group and three methoxyl groups thus permitting its representation by the partial formula, $C_{15}H_5O_2(CH_2O_2)(OMe)_3$. The association of four aromatic protons with four singlets (τ 2·37, τ 3·12, τ 3·17, and τ 3·37) required the presence of two pairs of *para*-related protons thus leading to two possible structures for milldurone, namely 6,7,2′-trimethoxy-4′,5′-methylenedioxyisoflavone or 2′,4′,5′-trimethoxy-6,7-methylenedioxyisoflavone. The former structure for milldurone (VIII) was established by its alkaline hydrogen peroxide oxidation. This yielded 2-hydroxy-4,5-dimethoxybenzoic acid identical with a synthetic specimen[29] and 6-methoxypiperonylic acid previously isolated by a similar degradation of ichthynone.[26]

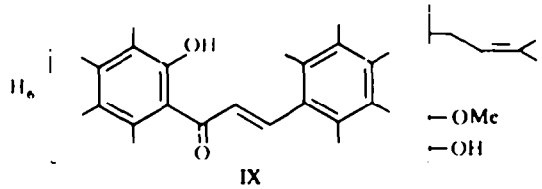
Compound $C_{21}H_{22}O_4$. The amount of this compound available for structural study was small and our opinion regarding its nature rests solely upon spectroscopic and mass spectrometric evidence. Acetylation gave a crystalline monoacetate and a presumed diacetate. The high resolution mass spectrum of the monoacetate established the molecular formula, $C_{23}H_{24}O_5$, and the presence of one exchangeable hydrogen atom was shown by deuteration studies. The natural product, $C_{21}H_{22}O_4$, and its monoacetate apparently contained an *o*-hydroxy-carbonyl function as these two

TABLE 2. UV ABSORPTION SPECTRA OF DURMILLONE, MILLDURONE, AND RELATED ISOFLAVONES
λ_{max} mμ (ε_{max}) in ethanol

Durmillone (IV)	230	(26,700)	255	(28,600)	334	(9700)
					347	(9000)
Jamaicin[25](V)	232	(30,500)	263	(34,700)	306	(14,500)
Ichthynone[26] (VI)	232	(33,600)	262	(24,300)	309	(14,100)
					331	(11,000)
					345	(9400)
Milldurone (VIII)	232	(20,800)	256	(13,800)	312	(16,500)
6,7-Dihydroxy-4′-methoxyisoflavone[28]	231	(24,550)	258	(33,110)	326	(13,490)
Afrormosin[30] (7-hydroxy 6,4′-dimethoxyisoflavone)			258	(23,440)	320	(10,000)
6,7,4′-Trimethoxyisoflavone[30]			261	(50,120)	320	(19,950)

[28] S. F. Dyke, W. D. Ollis, M. Sainsbury and J. S. P. Schwarz. *Tetrahedron* **20**, 1331 (1964)
[29] S. Rajagopalan, T. R. Seshadri and S. Varadarajan. *Proc. Ind. Acad. Sci.* **30A**, 265 (1949)
[30] T. B. H. McMurry and C. Y. Theng, *J. Chem. Soc.* 1491 (1960).

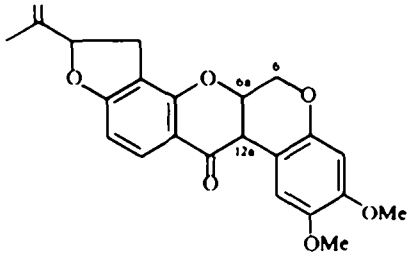
compounds showed ν_{CO} at 1640 cm^{-1} and a positive ferric chloride reaction; the presumed diacetate showed ν_{CO} at 1655 cm^{-1} and a negative reaction with ferric chloride. The UV spectrum of compound $C_{21}H_{22}O_4$ (λ_{max}: 234 and 373 mμ) was similar to that of 2',4'-dihydroxychalkone (λ_{max}: 242 and 370 mμ) and the expected hypsochromic shift was shown by the monoacetate, $C_{23}H_{24}O_5$ (λ_{max}: 223 and 362 mμ). This UV and IR evidence suggests that compound $C_{21}H_{22}O_4$ is a chalkone[19] and as the mass spectra suggest the presence of a methoxyl group and a C_5H_9-substituent a partial structure of the type IX may be tentatively proposed. Further evidence regarding the nature of this compound is not available.



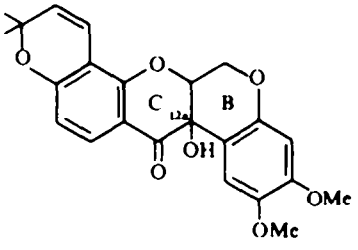
IX

Compounds of the rotenoid type isolated from M. dura

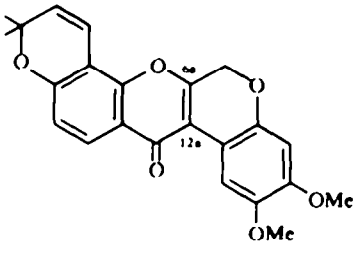
The known compounds of the rotenoid type[4] which have been isolated are rotenone (X), tephrosin (XI) in the optically active and racemic forms, and $6_a,12_a$-dehydrodeguelin (XII). The relation of these results to the general question of the natural occurrence of 12_a-hydroxyrotenoids is considered later.



X: Rotenone



XI: Tephrosin



XII: $6_a,12_a$-Dehydrodeguelin

Millettone.• This is a new rotenoid and unlike all the other natural rotenoids[4] it is the first to be recognized which contains no methoxyl groups. This, as it transpires,

• Our studies of millettone have been mentioned in another publication[31] but unfortunately millettone was mis-named munduserone.

[31] D. J. Adam, L. Crombie and D. A. Whiting, *J. Chem. Soc.* (C) 542 (1966).

is a particularly useful circumstance. Normally a complete interpretation of the NMR spectra of rotenoids[32] is not possible because the methoxyl signals interfere with the detailed interpretation of the ABCD system associated with the four protons in positions 6, 6_a, and 12_a (see formula X). The absence of methoxyl groups in millettone therefore permits a detailed analysis of its NMR spectrum and leads to a deduction of not only its constitution but also its relative configuration and conformation.

Millettone, $C_{22}H_{18}O_6$, showed a positive Durham test which suggested that it was a rotenoid and this was obviously supported by its UV spectrum [λ_{max}: 243 mμ (ε 18,500), 271 mμ (ε 22,200), 305 mμ (ε 12,700)] which is very similar to that of munduserone[33] [λ_{max}: 232 mμ (ε 16,100), 278 mμ (ε 14,700), 314 mμ (ε 7900).] Its IR spectrum showed a carbonyl band (ν_{CO} 1680 cm^{-1}) typical of rotenoids and isoflavanones bearing no 11- or 5-hydroxyl group respectively; for example, munduserone[33] shows ν_{CO} = 1676 cm^{-1}. Other IR absorption bands of millettone could be attributed to the carbon-carbon double bond (ν_{max} 1580 cm^{-1}) and *gem*-dimethyl group (ν_{max} 1391, 1379 cm^{-1}) of a 2,2-dimethylchromene system[20,25,26] and the bands (ν_{max} 1040 and 940 cm^{-1}) suggested the presence of a methylenedioxy group.[34]

The NMR spectrum of millettone (Fig. 1) clearly indicated the presence of a 2,2-dimethylchromene system,[20,21] a methylenedioxy group, four aromatic protons, and an ABCD system characteristic of rotenoids due to the four protons in position 6, 6_a, and 12_a (see XIII). The four aromatic protons were allocated to a pair which were *para*-related (τ 3·18 doublet* and τ 3·53 singlet) and their chemical shifts indicated[32] that they were in positions 1 and 4 of the rotenoid skeleton with oxygen atoms in positions 2 and 3 which were necessarily associated with the methylenedioxy group. The other pair of aromatic protons (τ 2·17 doublet, J = 8·5 c/s; τ 3·21 quartet, J = 8·5 and 0·7 c/s) formed an AB system characteristic of *ortho*-related protons; the low field proton could be placed in position 11 as it was deshielded by the adjacent carbonyl group and the chemical shift (τ 3·21) of the high field proton, indicated that it was adjacent to an oxygen atom which had to be the ether oxygen of the dimethylchromene system. The aromatic proton in position 10 clearly showed long range coupling (J = 0·7 c/s) with the 4'-olefinic proton (τ 3·21) of the dimethylchromene residue (two nonequivalent methyl groups, τ 8·55 and τ 8·61 singlets; AB system, τ 4·47 doublet J = 10·1 c/s and τ 3·21 quartet J = 10·1 and 0·7 c/s). The long range coupling over five bonds between the aromatic proton in position 10 and the 4'-olefinic proton (see XIII) involves a geometrical situation identical with that observed in other similar cases.[35]

Thus consideration of the NMR spectrum of millettone led to the constitutional proposal XIII and this was confirmed by the following reactions. Catalytic hydrogenation of millettone (XIII) gave dihydromillettone (XIV). Both millettone (XIII) and dihydromillettone (XIV) were transformed into their 6_a,12_a-dehydroderivatives

* The origin of this additional coupling (J = 1·0 c/s) is indicated in Fig. 3.

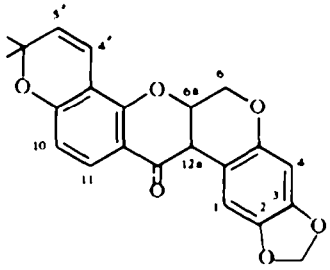
[32] I. Crombie and J. W. Lown, *Proc. Chem. Soc.* 299 (1961); *J. Chem. Soc.* 775 (1962).

[33] N. Finch and W. D. Ollis, *Proc. Chem. Soc* 176 (1960).

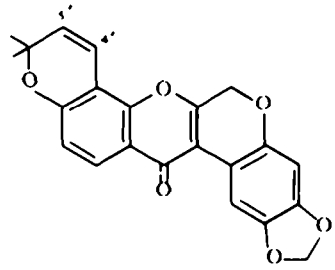
[34] L. Briggs, L. Colebrook, H. Fales and W. C. Wildman, *Analyt. Chem.* 29, 904 (1957).

[35] D. D. Elleman and S. L. Manatt, *J. Chem. Phys.* 36, 2346 (1962); J. A. Elvidge and R. G. Foster, *J. Chem. Soc* 590 (1963); M. Martin-Smith, S. T. Reid and S. Sternhell, *Tetrahedron Letters* 2393 (1965) and Refs there cited; S. Sternhell, *Rev. Pure and Appl. Chem.* 14, 15 (1964).

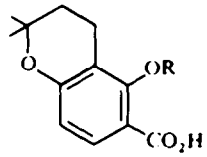
(XV and XVI respectively) and oxidation of the latter gave dihydro-β-tubaic acid (XVII).



XIII: Millettone
XIV: (Single bond at 4',5')



XV
XVI: (Single bond at 4',5')



XVII: R = H
XVIII: R = Me

The non-equivalence of the two methyl groups (τ 8·55 and 8·61) and the two protons of the methylenedioxy group[36] (τ 4·09 and 4·14 doublets J = 1·4 c/s) is due to the chirality of the rotenoid skeleton. The coupling exhibited by the methylenedioxy protons matches the observations made by Crabb and Cookson[37] of the coupling constants shown by methylenedioxy groups in 5-membered rings ($J = 0 \simeq 2$ c/s) as compared with 6-membered rings (J = 6 c/s).

Consideration could now be given to the analysis of the signals due to the four protons in positions 6, 6_a, and 12_a (see XIII). It is apparent that they form an ABCD system and first order analysis readily gave chemical shifts and coupling constants. A more careful examination revealed two small long range coupling constants (J_{CD} and J_{DE}, see Figs. 2 and 3) and using the values for coupling constants and chemical shifts given in Fig. 3 for the ABCDE system it was possible to obtain a good match between computed* and observed spectra (Fig. 2). Signs have not been allocated to the coupling constants as these have little effect upon the appearance of the spectrum. The coupling constants establish that millettone has the stereoformula (see Fig. 2), or its mirror image, and demonstrate that it has a B/C *cis* ring fusion in accord with that of the other rotenoids.[38, 39]

* We thank Mr. B. Price for computing the theoretical spectrum.

[36] I. R. C. Bick, J. Harley-Mason, N. Sheppard and M. J. Vernengo, *J. Chem. Soc.* 1896 (1961); R. F. Manske, K. H. Shin, A. R. Battersby and D. F. Shaw, *Canad. J. Chem.* **43**, 2183 (1965).

[37] T. A. Crabb and R. C. Cookson, *Tetrahedron Letters* No. 12, 679 (1964); R. C. Cookson, T. A. Crabb, J. J. Frankel and J. Hudek, *Tetrahedron* Supplement No. 7, 355 (1966).

[38] G. Büchi, L. Crombie, P. J. Godin, J. S. Kaltenbronn, K. S. Siddalingaiah and D. A. Whiting, *J. Chem. Soc.* 2843 (1961).

[39] C. Djerassi, W. D. Ollis and R. C. Russell, *J. Chem. Soc.* 1448 (1961).

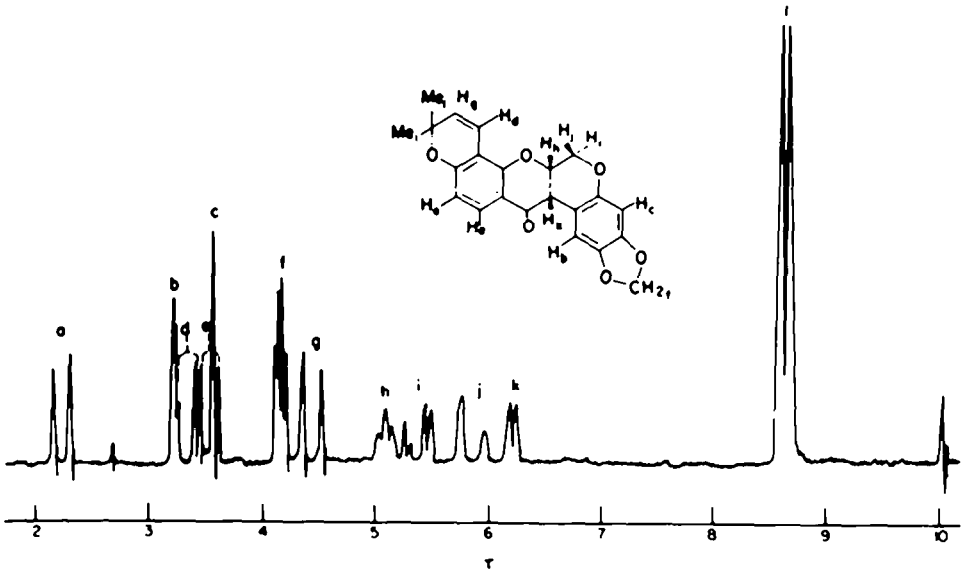


FIG. 1 Nuclear magnetic resonance spectrum of millettone (XIX) ($CDCl_3$ solution)

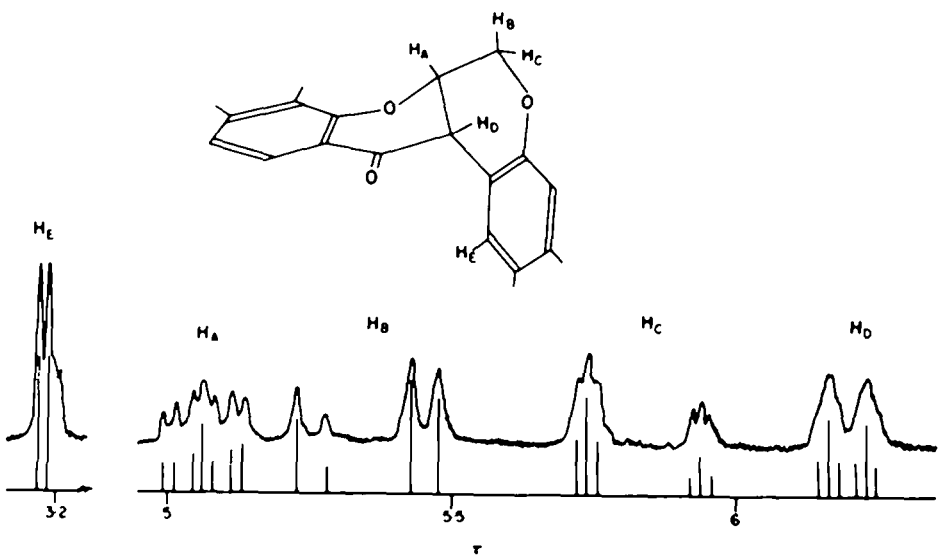
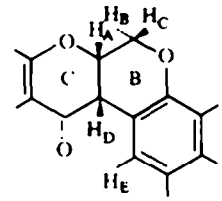


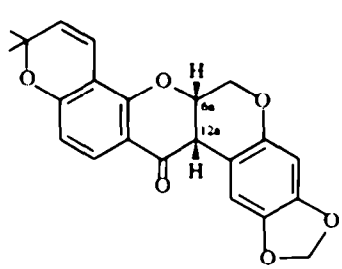
FIG. 2 The stereochemistry of millettone and the analysis of the ABCDE system of its NMR spectrum ($CDCl_3$ solution)



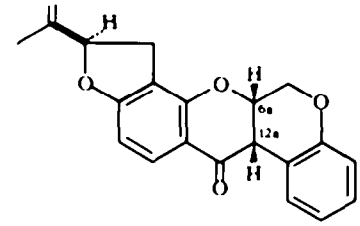
Chemical shifts τ		Coupling constants c/s	
H_A	5·11	J_{AB}	3·2
H_B	5·42	J_{AC}	1·1
H_C	5·87	J_{AD}	4·0
H_D	6·25	J_{BC}	12·2
H_E	3·18	J_{CD}	1·1
		J_{DE}	1·0

FIG. 3 The chemical shifts and coupling constants deduced by the analysis of the spectrum given in Fig. 2. The long range couplings J_{CD} and J_{DE} account for additional coupling seen in the signals of protons C, D, and E.

The absolute configuration XIX for (−)-millettone was now established by a comparison of its ORD curve (Fig. 4) with the ORD curve (Fig. 5) of rotenone (XX) of proven absolute stereochemistry.[38] In our earlier studies[39] of the absolute stereochemistry of natural rotenoids, it was shown that they all showed a positive Cotton effect to be associated with the (6_a-S, 12_a-S) absolute configuration with, of course, a B/C *cis* fusion. Through the kindness of Professor C. Djerassi (Stanford University) it was later possible to determine the ORD curves of millettone and rotenone (Figs 4 and 5) down to lower wavelengths and it is clear that the positive Cotton effect described previously[39] is associated with negative Cotton effects *of much larger amplitudes* at shorter wavelengths. These ORD curves show the appropriate relation to the corresponding CD curves and the UV absorption maxima of millettone (XIX) and rotenone (XX) (see Figs. 4 and 5) and confirms the (6_a-S, 12_a-S) absolute configuration of millettone.



XIX: (−)-Millettone



XX: (−)-Rotenone

It is possible that millettone may be a fairly widely distributed rotenoid since we have found it to be also present in plants belonging to two other genera, namely *Piscidia erythrina*[40] and *Lonchocarpus rugosus*.[41]

[40] C. P. Falshaw, W. D. Ollis, J. A. Moore and K. Magnus, *Tetrahedron* Supplement No. 7, 333 (1966).
[41] B. Blessington (University of Sheffield). Unpublished observation

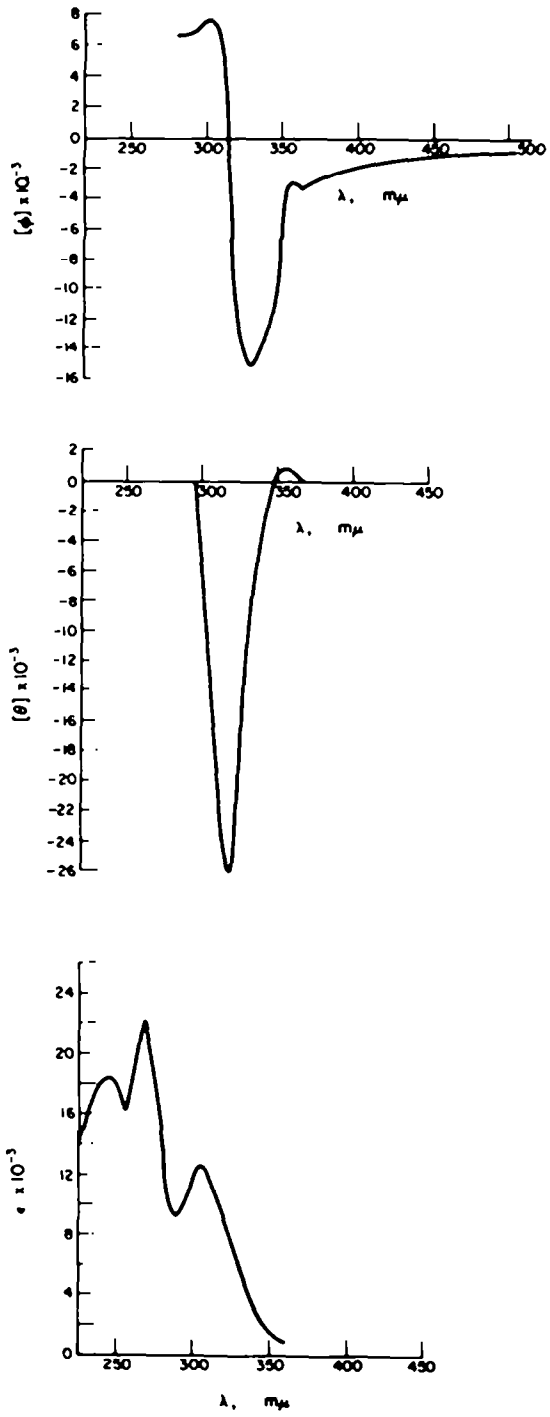


FIG. 4 Optical rotatory dispersion and circular dichroism curves of (−)-millettone (XIX) and its ultraviolet spectrum.

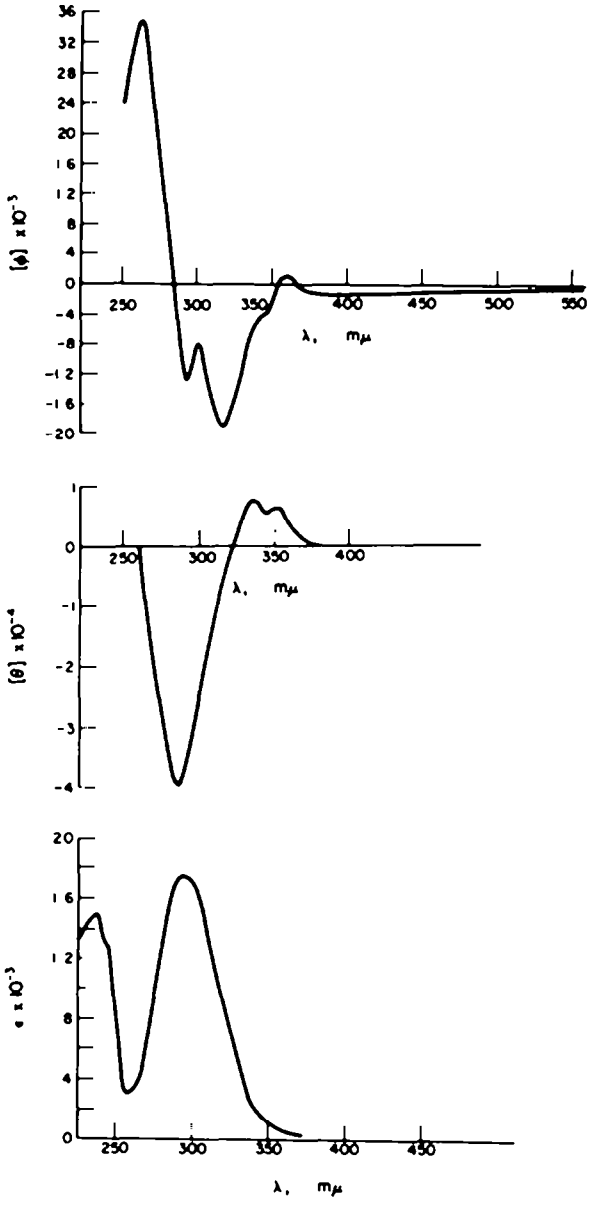


FIG 5 Optical rotatory disperson and circular dichroism curves of (−)-rotenone (XX) and its ultraviolet spectrum.

Millettosin. Only a small quantity of this compound was isolated so initially a reasonable constitution was proposed on the basis of physical data and then this was confirmed by the partial synthesis of its racemate.

The mass spectrum of millettosin showed a parent peak at *m/e* 394 which could correspond either with the molecular formula $C_{23}H_{22}O_6$ (an isomer of rotenone)

or $C_{22}H_{18}O_7$ (a hydroxy derivative of millettone). The latter possibility was immediately attractive because the IR spectrum of millettosin was almost superimposable upon that of millettone and showed carbonyl absorption (ν_{max} 1674 cm^{-1}) and bands characteristic of a 2,2-dimethylchromene residue[20, 25, 26] (ν_{max} 1639, 1390, 1380 cm^{-1}) and a methylenedioxy group[34] (ν_{max} 1040, 940 cm^{-1}). Millettosin like tephrosin (XI) gave a negative Durham test and no coloration with ferric chloride. This information suggested that millettosin was 12$_a$-hydroxymillettone and this received striking support from a comparison of the mass spectra of millettosin and tephrosin (XI) which showed a number of common or corresponding fragment ions (see Fig. 6). The fragmentation patterns of these 12$_a$-hydroxyrotenoids are similar to those which have been observed for rotenoids.[42] Acid-catalysed dehydration of millettosin gave 6$_a$,12$_a$-dehydromillettone (XV) but this dehydration was not observed during its mass spectrometric study.

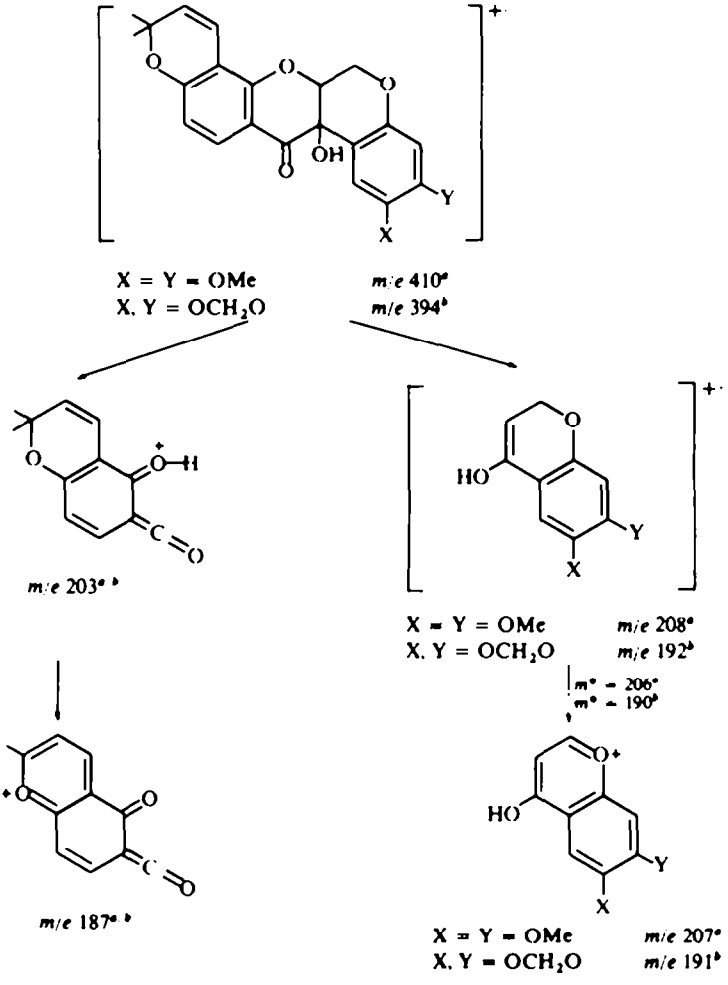
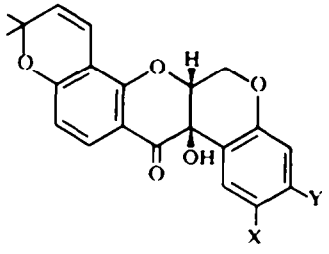


FIG. 6 The mass spectra of tephrosin and millettosin. The ions from tephrosin are labelled [a] and those from milletosin [b].

[42] R. I. Reed and J. M. Wilson, *J. Chem. Soc.* 5949 (1963).

The constitution 12$_a$-hydroxymillettone was therefore proposed for millettosin and this was confirmed by the transformation of millettone into the racemic α-ketol which was shown to correspond with natural (−)-millettosin. It has been shown in a definitive study by Crombie and Godin[17] that aerial oxidation of deguelin gives (±)-tephrosin and (±)-isotephrosin and their configurations were established as tephrosin (XI; B/C *cis*) and isotephrosin (XI; B/C *trans*). Tephrosin shows intramolecular hydrogen bonding (ν_{CO} 1675 and ν_{OH} 3509) and comparison with (−)-millettosin (ν_{CO} 1674 and ν_{OH} 3508 cm^{-1}) shows that it also has the B/C *cis* configuration.

Comparison of the ORD curves of (−)-millettosin and (−)-tephrosin shows that they have the corresponding stereochemical configurations XXI and XXII, but it is not known whether these formulae, or their enantiomeric forms, represent their actual absolute configurations. As far as we are aware this is the first example of the isolation of *optically active* 12$_a$-hydroxyrotenoids from natural sources.[4b]



XXI: X, Y = OCH_2O (−)-Millettosin
XXII: X = Y = OMe (−)-Tephrosin

The natural occurrence of 12$_a$*-hydroxyrotenoids and* 6$_a$,12$_a$*-dehydrorotenoids*

The question whether 12$_a$-hydroxyrotenoids are genuine natural products or whether they are artefacts produced by oxidation of rotenoids either after the plant material was collected or during the isolation procedure has been considered on several occasions.[4b, 16, 17, 18] Tephrosin (XI) has been isolated from a number of plants including *Lonchocarpus nicou*,[43] *Derris elliptica*,[43] *Tephrosia vogelii*,[43, 46] and *Tephrosia obovata*.[47] None of these reports refers to the isolation of optically active tephrosin and in those cases where it was examined the absence of optical activity is noted. In some of these cases alkaline extraction was used during the isolation and in view of the known ease of oxidation of deguelin[17] to *racemic* tephrosin and *racemic* isotephrosin in alkaline solution, this supports the view[4b] that (±)-tephrosin is an artefact. However, the isolation of (−)-millettosin (XXI) and (−)-tephrosin (XXII) from *M. dura* which is now reported does suggest that they may well be natural products. Comparative chromatographic examination of the initial extracts supported their presence in the plant material, but the isolation of (±)-tephrosin from the hot methanol extract should be noted.

In view of the established ease of dehydration of 12$_a$-hydroxyrotenoids, 6$_a$,12$_a$-dehydrorotenoids could well be artefacts. As well as its isolation from *M. dura*,

[43] F. P. Clark, *J. Am. Chem. Soc* **53**, 729 (1931)
[44] M. Hanriot, *C. R. Acad. Sci., Paris* **144**, 150 (1907)
[45] R. R. le G. Worsley, *Ann. Appl. Biol.* **21**, 649 (1934).
[46] S. Rangaswami and B. V. Rama Sastry, *Ind. J. Pharm.* **18**, 339 (1956); *Chem. Abstr* **51**, 2234 (1957)
[47] Yuh-Lin Chen and Hong-Yen Hsu, *Yakugaku Zasshi* **78**, 198 (1958); *Chem. Abstr.* **52**, 9520 (1958).

6$_a$, 12$_a$-dehydrodeguelin (XII) has been isolated from *Tephrosia vogelii*[46] and from *Derris* root.[48] 6$_a$,12$_a$-Dehydromillettone has been isolated from *Piscidia erythrina*.[40]

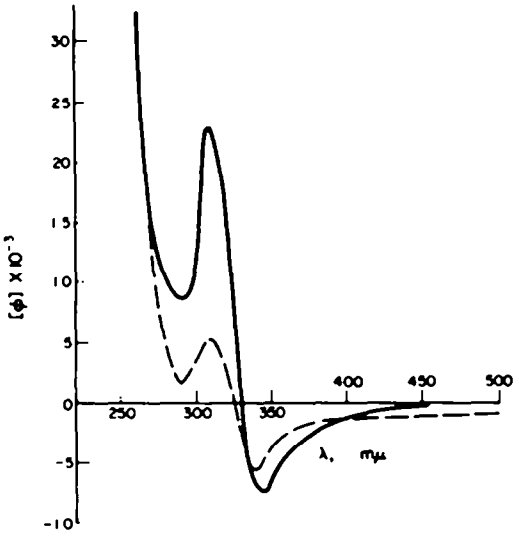


FIG. 7 Optical rotatory dispersion curves of (−)-millettosin (XXI) (full line) and (−)-tephrosin (XXII) (broken line) isolated from *M. dura*.

EXPERIMENTAL

NMR spectra were determined on $CDCl_3$ solns using a Varian A-60 spectrometer; TMS was used as the internal standard. Only significant bands from IR spectra are quoted.

M.ps were determined using a Kofler hot stage microscope and are uncorrected.

Separations by column chromatography were carried out using Hopkin and Williams' MFC grade silica. Merck's Kieselgel G was used for thick and thin layer chromatography (TLC). During isolation processes the appropriate combination of fractions was determined by examination of their IR spectra and TLC behaviour. Thin layer chromatograms were examined under UV illumination and by spraying with (a) ethanolic ferric chloride soln (1 %) and (b) ceric sulphate in sulphuric acid soln (2 %) followed by heating.

Extraction of the seeds of Millettia dura (Dunn)

The ground seeds (1 Kg) were consecutively extracted with the following solvents and yielded the indicated fractions.

Two extractions (24 hr periods) with cold light petroleum (b.p. 60–80°) (2 × 1·5 l. portions) yielded fraction A (208 + 36 g). This was shown to be fatty material and was not further examined.

Four extractions (24 hr periods) with cold ether (4 × 1·5 l. portions) yielded fraction B (18·8 + 9·8 + 4·4 + 2·4 g).

One continuous extraction (10 days) with hot ether yielded fraction C (9·2 g).

Two continuous extractions (24 hr periods) with hot MeOH yielded fraction D (159 + 12 g).

Examination of fraction B

Isolation of durlettone (I), *durmillone* (IV), *milldurone* (VIII), *compound* $C_{21}H_{22}O_4$ (IX), (−)-*millettone* (XIX), (−)-*millettosin* (XXI), (−)-*rotenone* (XX), (−)-*tephrosin* (XXII), and 6$_a$,12$_a$-*dehydrodeguelin* (XII). The combined material (fraction B, 35·4 g) from the cold ether extraction was chromatographed using successively benzene (B_1 and B_2) benzene–$CHCl_3$ mixtures (B_3 and B_4), $CHCl_3$ (B_5 and B_6), AcOEt (B_7), and MeOH (B_8) as eluting solvents. Appropriate fractions (IR spectra and TLC) were combined and this yielded seven fractions (B_1 – $_7$).

B_1 (7 g) was a yellow oil of a fatty nature and it was not further examined.

B_2 (1·68 g) was rechromatographed and fractional elution with benzene first yielded a *compound* (320 mg)

[48] M. Miyano, T. Nishikubo and M. Matsui, *Chem. Ber.* 93, 1746 (1960).

which was crystallized from MeOH giving white plates, m.p. 74°. (Found: C, 77·18; H, 12·9%). This compound was aliphatic in type and was not examined further. Later fractions gave *millettone* (620 mg).

B_3 (860 mg) was separated by *thick* layer chromatography (benzene-$CHCl_3$ solvent 50:50) and this yielded a number of bands which by separation, extraction, and crystallization yielded *durlettone* (15 mg), (−)-rotenone (30 mg), *compound* $C_{21}H_{22}O_6$ (20 mg), and (−)-*millettosin* (12 mg).

B_4 (4·75 g) was separated by multiple chromatography and fractional crystallization. This yielded durlettone (37 mg), (−)-rotenone (293 mg), (−)-tephrosin (290 mg), and 6$_a$,12$_a$-dehydrodeguelin (10 mg).

B_5 (4·15 g) similarly yielded *durmillone* (37 mg), (−)-rotenone (293 mg), (−)-tephrosin (290 mg), and 6$_a$,12$_a$-dehydrodeguelin (10 mg).

B_6 (10·52 g) similarly yielded (−)-rotenone (102 mg), durmillone (199 mg), milldurone (111 mg), and (−)-tephrosin (30 mg).

B_7 (200 mg) was examined but nothing useful could be isolated.

Examination of fraction C

Isolation of durlettone (I), *durmillone* (IV), *milldurone* (VIII), (−)-*millettone* (XIX), (−)-*rotenone* (XX), (−)-*tephrosin* (XXII), *and* 6$_a$,12$_a$-*dehydrodeguelin* (XII). Fraction C (9·2 g) obtained by extraction with hot ether was triturated with benzene and the soluble portion (8·65 g) was fractionated chromatographically using benzene (C_1, C_2, and C_3), benzene-$CHCl_3$ mixtures (C_4, C_5, and C_6), $CHCl_3$ (C_7), $CHCl_3$ AcOEt mixtures (C_8) and AcOEt MeOH mixtures (C_9). Appropriate fractions (IR spectra and TLC) were combined and this yielded 9 main fractions (C_{1-9}) which were examined as for fractions B_{1-7}.

C_1 (305 mg) was a yellow oil of a fatty nature. It was not examined further.

C_2 (144 mg) gave (−)-millettone (80 mg).

C_3 (680 mg) gave (−)-rotenone (203 mg) and durlettone (10 mg).

C_4 (1·07 g) gave 6$_a$,12$_a$-dehydrodeguelin (70 mg) and durmillone (60 mg).

C_5 (1·03 g) contained several compounds which were chromatographically indicated to be rotenone, 6$_a$,12$_a$-dehydrodeguelin, durmillone, milldurone, and tephrosin. The separation of C_5 was not pursued.

C_6 (163 mg) gave milldurone (25 mg).

C_7 (413 mg), C_8 (655 mg), and C_9 contained mainly tephrosin and were not examined further.

Examination of fraction D

Isolation of durlettone (I), *durmillone* (IV), (−)-*millettone* (XIX), (−)-*rotenone* (XX), (±)-*tephrosin* (XI), *and* 6$_a$,12$_a$-*dehydrodeguelin* (XII). The combined material (fraction D, 171 g) obtained by extraction with hot MeOH was rather intractable so only that material which moved during thick layer chromatography using $CHCl_3$ as solvent was examined. Thick plates (250) yielded two bands (UV light) and their extraction gave fractions D_1 (320 mg) and D_2 (70 mg).

D_1 yielded durlettone (5 mg), durmillone (7 mg), (−)-millettone (20 mg), (−)-rotenone (4 mg), and 6$_a$,12$_a$-dehydrodeguelin (1 mg).

D_2 gave (±)-tephrosin (46 mg).

The Identification of the Extractives of M. dura

Extraction of the seeds (1 Kg) of *M. dura* as indicated above yielded the following compounds. The total quantities of pure substances that were isolated are given in parentheses: durlettone (I, 54 mg), durmillone (IV, 303 mg), milldurone (VIII, 136 mg), compound $C_{21}H_{22}O_6$ (IX, 20 mg), (−)-millettone (XIX, 720 mg), (−)-millettosin (XXI, 12 mg), (−)-rotenone (XX, 632 mg), (−)-tephrosin (XXII, 320 mg), (±)-tephrosin (XI, 46 mg), and 6$_a$,12$_a$-dehydrodeguelin (XII, 81 mg). The information regarding these compounds is now summarized.

Durlettone (I), m.p. 137°, was obtained by crystallization from MeOH. (Found: C, 74·83; H, 5·99; OMe, 9·92. $C_{20}H_{17}O_3$·OMe requires: C, 75·04; H, 5·99; OMe, 9·22%). λ_{max} (in EtOH) 236 mμ infl. (ε 21,100), 260 mμ (ε 26,400), 297 mμ infl. (ε 9990). ν_{max} (in $CHCl_3$) 1640, 1630, and 1610 cm^{-1}. NMR spectrum (in $CDCl_3$) τ 2·00 singlet (2-H); τ_X 1·70, τ_A 2·93, τ_B 3·07 (ABX system J_{AX} = 9 c/s, J_{AB} = 2 c/s, 5H, 6H, 8H); τ_A 2·41, τ_X 2·94 (A_2X_2 system J_{AX} = 9 c/s, 2H, 3'H, 5'H, 6'H); τ 6·05, singlet (OCH_3); τ 4·43 triplet (J = 6·5 c/s, CH_2—CH—CMe_2); τ 5·39 doublet (J = 6·5 c/s, CH_2—CH=CMe_2); τ 8·19 broad singlet [CH_2—CH=$C(CH_3)_2$].

Durmillone (IV), m.p. 171–172°, as fine colourless needles from MeOH. (Found: C, 70·16; H, 4·95; OMe, 9·31. $C_{21}H_{13}O_5$·OMe requires: C, 69·83; H, 4·79; OMe, 8·91%.) λ_{max} (in EtOH) 230 mμ (ε 26,700), 255 mμ (ε 28,600), 334 mμ (ε 9700), 347 mμ (ε 9000). ν_{max} (in $CHCl_3$) 1640, 1040, 940 cm^{-1}. NMR spectrum

(in $CDCl_3$) [τ 2·08 singlet (2-H); τ 2·43 singlet (5-H); τ 2·79 3·26 multiplet (ABX system, H_A τ 3·14, H_B τ 3·02, H_X τ 2·89, J_{AB} = 8·0 c.s, J_{BX} = 1·6 c.s, J_{AX} = 0·6 c/s); τ 3·19 doublet and τ 4·27 doublet (J = 10 c/s, AB system, —O—CMe$_2$—CH=CH—); τ 4·02 singlet (—O—CH$_2$—O—); τ 6·04 singlet (—OCH$_3$); τ 8·45 singlet (O—CMe$_2$—CH=CH)].

Milldurone (VIII), m.p. 233–234°, as fine colourless crystals from MeOH. (Found: C, 63·87; H, 4·69. $C_{19}H_{16}O_5$ requires: C, 64·04; H, 4·53%.) λ_{max} (in EtOH) 232 mμ (ε 20,800), 256 mμ (ε 13,800) 312 mμ (ε 16,500). ν_{max} (in $CHCl_3$) 1640, 1040 and 940 cm^{-1}. NMR spectrum (in $CDCl_3$) [τ 2·10 singlet (2-H); τ 2·37 singlet (5-H); τ 3·12 singlet (8-H); τ 3·17 singlet (6'-H); τ 3·37 singlet (3'-H); τ 4·05 singlet (O—CH$_2$—O); τ 6·02 singlet (6-OCH$_3$); τ 6·02 singlet (7-OCH$_3$); τ 6·27 singlet (2'-OCH$_3$)].

Compound $C_{21}H_{22}O_4$ (IX) was obtained as a yellow oil. [Found: M (mass spectrum) 338. $C_{21}H_{22}O_4$ requires M: 338.] λ_{max} (in EtOH) 234 mμ (ε 18,900), 373 mμ (ε 26,100). ν_{max} (in $CHCl_3$) 3650, 3320 and 1640 cm^{-1}. It gave a dark brown coloration with ethanolic ferric chloride.

Millettone (XIX), m.p. 180–181°, was obtained as cream needles from methanol (Found: C, 69·67; H, 4·85. $C_{22}H_{18}O_6$ requires: C, 69·84; H, 4·79%.) λ_{max} (in EtOH) 243 mμ (ε 18,500), 271 mμ (ε 22,200), 305 mμ (ε 12,700). ν_{max} (in $CHCl_3$) 1680, 1640, 1610, 1580, 1391, 1379, 1040, 940 cm^{-1}. NMR spectrum (in $CDCl_3$) [τ 2·17 doublet (J = 8·5 c.s, 11-H); τ 3·18 doublet (J = 10 c.s, 1-H); τ 3·21 quartet (J = 10·1 and 0·7 c.s, O—CMe$_2$—CH=CH—); τ 3·47 quartet (J = 8·5 c.s and 0·7 c.s, 10-H); τ 3·53 singlet (4-H); τ 4·09 doublet and τ 4·14 doublet (J = 1·4 c.s, AB system, O—CH$_A$H$_B$—O); τ 4·47 doublet (J = 10·1 c.s, —O—CMe$_2$—CH=CH—); τ 5·0–6·7 multiplet (ABCD system, H_A τ 5·11, H_B τ 5·42, H_C τ 5·87, H_D τ 6·25, J_{AB} = 3·2 c.s, J_{AC} = 1·1 c.s, J_{AD} = 4·0 c.s, J_{BC} = 12·2 c.s with additional long range coupling as described in Fig. 3); τ 8·55 singlet and τ 8·61 singlet (O—CMe$_2$—CH—CH—)] ORD in dioxan (c 0·8, 600–270 mμ): $[\phi]_{589}$ −380, $[\phi]_{390}$ −3020, $[\phi]_{331}$ −15,120, $[\phi]_{320}$ −10,580, $[\phi]_{303}$ +7560. CD in dioxan (c 4·3, 400–330 mμ; 0·215, 330–320 mμ; 0·086, 320–280 mμ): $[\theta]_{355}$ +830, $[\theta]_{320}$ −26,110. Millettone gave a positive Durham test.

(−)-Millettosin (XXI) was obtained as a pale yellow oil which gave no coloration with ethanolic ferric chloride and a negative result in the Durham test. [Found: M (mass spectrum) 394. $C_{22}H_{18}O_7$ requires: 394.] λ_{max} (in EtOH) 215 mμ (ε 13,500), 233 mμ (ε 19,300), 251 mμ (ε 20,900), 274 mμ (ε 23,900), 310 mμ (ε 10,100). ν_{max} (in $CHCl_3$) 3508, 1674, 1639, 1590, 1390, 1380, 1040, 940 cm^{-1}. ORD in MeOH (c 12·59, 500–250 mμ). $[\phi]_{430}$ −350, $[\phi]_{345}$ −7410, $[\phi]_{309}$ +22,770, $[\phi]_{290}$ +8550, $[\phi]_{250}$ +50,430.

Rotenone (XX), m.p. 163–164° (lit. m.p. 167–168°),[46] was obtained as light yellow platelets by crystallization from MeOH. ν_{max} (in $CHCl_3$) 1670, 1610 and 1510 cm^{-1}. ORD in dioxan $[\phi]_{589}$ −410, $[\phi]_{398}$ −1140, $[\phi]_{358}$ +1380, $[\phi]_{353}$ 0, $[\phi]_{342}$ −4530, $[\phi]_{317}$ −18,910, $[\phi]_{302}$ −7800, $[\phi]_{292}$ −12,610, $[\phi]_{264}$ +34,670. CD in dioxan (c 10, 400–330 mμ; 2, 330–310 mμ; 0·08, 310–260 mμ): $[\theta]_{354}$ +6118, $[\theta]_{345}$ +5320, $[\theta]_{336}$ +7448, $[\theta]_{285}$ −39,900.

(−)-Tephrosin (XXII) was obtained as a colourless oil which gave no coloration with ethanolic ferric chloride and a negative result in the Durham test. It could not be crystallized but was precipitated as an amorphous solid by addition of cyclohexane to its solution in ether. [Found: M (mass spectrum) 410. C, 67·61; H, 5·68. Calc. for $C_{23}H_{22}O_7$: M, 410; C, 67·31; H, 5·40%.] λ_{max} (in EtOH) 237 mμ (ε 16,100), 251 mμ (ε 16,500), 272 mμ (ε 19,000), 300 mμ (ε 10,100), 317 mμ infl. (ε 8300). ν_{max} (in $CHCl_3$), 3500, 1670, 1640, 1385, 1370 cm^{-1}. $[\alpha]_D^{23}$ −113° (c 7·8 in C_6H_6). ORD in MeOH: $[\phi]_{500}$ −900, $[\phi]_{340}$ −5660, $[\phi]_{309}$ +5290, $[\phi]_{289}$ +750, $[\phi]_{250}$ +52,100.

(±)-Tephrosin (XI), m.p. 198° (lit. m.p. 198–199°),[17] was obtained by crystallization from MeOH. $[\alpha]_D^{23}$ 0 (in benzene). λ_{max} (in EtOH) 211 mμ (ε 21,900), 239 mμ (ε 17,900), 253 mμ (ε 18,100), 273 mμ (ε 22,600), 302 mμ (ε 8300), 315 mμ infl. (ε 8200). Its IR spectrum (in $CHCl_3$) was identical with that of (−)-tephrosin. NMR spectrum (in $CDCl_3$) [τ 2·27 doublet and 3·53 doublet (J = 8·5 c.s, AB system, 11-H and 10-H); τ 3·38 doublet (J = 10 c.s, —O—CMe$_2$—CH=CH—); τ 3·40 singlet (1-H); τ 3·52 singlet (4-H); τ 4·46 doublet (J = 10 c.s, —O—CMe$_2$—CH=CH—); τ 5·42 broad singlet (O—CH—CH$_2$—O); τ 5·44 broad singlet (removed on deuteration, —OH); τ 6·20 singlet (OCH$_3$); τ 6·27 singlet (—OCH$_3$); τ 8·55 singlet* and τ 8·62 singlet* (O—CMe$_2$—CH=CH)].

6$_a$,12$_a$-Dehydrodeguelin (XII) was obtained as fine yellow needles, m.p. 229–231°, (lit. m.p. 233°)[48] from MeOH. λ_{max} (in EtOH) 238 mμ (ε 29,800), 259 mμ (ε 30,000), 275 mμ (ε 28,700), 315 mμ (ε 15,600).

* Equivalent Me groups, τ 8·68, have been previously reported for (+)-tephrosin,[32] but magnetic non-equivalence is not unexpected.

ν_{max} (in $CHCl_3$) 1637, 1385, 1372 and 1114 cm^{-1}. It was identical (m.p., UV, and IR spectra) with an authentic sample kindly supplied by Professor M. Miyano.[49]

Formation of 4'-hydroxy-7-methoxyisoflavone from durlettone (I). A soln of durlettone (19 mg) in glacial AcOH (0·3 ml) and conc HCl (0·05 ml) was heated under reflux for 1 hr, cooled, poured into water (5 ml) and the ppt (17·5 mg) collected. Recrystallization from EtOH gave III (15 mg) as fine white needles, m.p. 221–224° (lit. m.p. 218–220°)[50] identical (mixed m.p. and IR spectrum) with an authentic specimen.[50]

Formononetin (7-hydroxy-4'-methoxyisoflavone). The following method is more satisfactory than that given in the literature.[51] 4-Methoxybenzyl-2,4-dihydroxyphenyl ketone[52] (4·5 g), ethyl orthoformate (75 ml), anhyd pyridine (150 ml), and piperidine (3 ml) were heated under reflux for 5 hr, cooled, and poured on to ice and 2N HCl. Extraction with $CHCl_3$ yielded an oil which crystallized, and crystallization from EtOH gave formononetin (3·02 g; 65%) as fine white needles, m.p. 254° (lit. m.p. 257°).[51]

Daidzein (7,4'-dihydroxyisoflavone). The following method is more satisfactory than that given in the literature.[50] Formononetin (1 g) was heated under reflux with a soln of HBr in glacial AcOH (50 ml; 22·5% HBr). After 24 hr the soln was cooled and poured into water (500 ml). The ppt was dissolved in AcOEt, extracted into Na_2CO_3 aq (5%), and this on acidification gave the product which was crystallized from EtOH giving daidzein (815 mg; 85%) as cream needles, m.p. 318° (lit. m.p. 320–322°).[50]

Durlettone (I). 4'-Hydroxy-7-methoxyisoflavone (300 mg, prepared by partial methylation of daidzein[50]) was dissolved in anhyd acetone (10 ml) and heated under reflux with anhyd K_2CO_3 (600 mg) and γγ-dimethylallyl bromide[53] (173 mg). After 4½ hr the mixture was poured into water (40 ml) and the acetone removed under diminished pressure. Ether extraction followed by crystallization from MeOH gave durlettone (290 mg), m.p. 138°, identical (UV and IR spectra) with the natural product.

7-(γγ-Dimethylallyloxy)-4'-methoxyisoflavone (II). As in the preceding experiment, formononetin (400 mg) gave *7-(γγ-dimethylallyloxy)-4'-methoxyisoflavone* (400 mg) as white plates, m.p. 138°, from MeOH. The mixed m.p. with durlettone was depressed. (Found: C, 74·72; H, 5·98; OMe, 9·50. $C_{20}H_{18}O_3 \cdot OMe$ requires: C, 75·04; H, 5·99; OMe, 9·22%.) λ_{max} (in EtOH) 211 mμ (ε 27,800), 240 mμ (ε 31,500), 252 mμ (ε 34,200), 262 mμ (ε 34,500), 299 mμ (ε 14,600). ν_{max} (in $CHCl_3$) 1650, 1630 cm^{-1}.

Tetrahydrodurmillone. Hydrogenation of durmillone (63 mg) in EtOH with platinic oxide catalyst (5 mg) for 4 hr at room temp and atm press. gave an oil (65 mg) after filtration and removal of the EtOH. This oil was purified by TLC (benzene-$CHCl_3$ solvent, 25:75). Extraction of the band which showed a purple fluorescence under UV light gave *tetrahydrodurmillone* (51 mg) as colourless prisms, m.p. 215°, from MeOH. (Found: C, 69·25; H, 5·78. $C_{22}H_{22}O_6$ requires: C, 69·10; H, 5·80%.) λ_{max} (in EtOH) 219 mμ (ε 18,200), 241 mμ (ε 15,600), 290 mμ (ε 15,900), 344 mμ (ε 7900). ν_{max} (in $CHCl_3$) 1660 cm^{-1}. NMR spectrum (in $CDCl_3$) [τ 2·75 singlet (5-H); τ 3·20 singlet (3 protons, 2'-H, 3'-H, 6'-H); τ 4·05 singlet (O—C$\underline{H}_2$–O); τ 5·34 doublet (J = 7 c.s., · O C$\underline{H}_2$ CHAr CO); τ 6·13 singlet (OC$\underline{H}_3$); τ 6·18 triplet (J = 7 c.s., O—CH$_2$ –C$\underline{H}$Ar –CO); τ 7·25 triplet (J = 7 c.s., C$\underline{H}_2$ CH$_2$– CMe$_2$ –O); τ 8·18 triplet (J = 7 c.s., CH$_2$ C$\underline{H}_2$ CMe$_2$ O—); τ 8·59 singlet (CM$\underline{e}_2$– O)]

3',4'-Dihydrodurmillone. Durmillone (30 mg) in EtOH (30 ml) was hydrogenated (10% Pd-C catalyst; 10 mg) for 5 hr at room temp and atm press. Filtration through kieselguhr and evaporation gave an oil (30 mg) which was crystallized from MeOH giving *3',4'-dihydrodurmillone* (25 mg) as white prisms, m.p. 226–227°. (Found: C, 69·25; H, 5·58. $C_{22}H_{20}O_6$ requires: C, 69·46; H, 5·30%.) λ_{max} (in EtOH), 225 mμ (ε 29,200), 267 mμ (ε 20,300), 296 mμ (ε 16,300), 324 mμ (ε 12,400). ν_{max} (in $CHCl_3$) 1640 cm^{-1}.

Oxidation of 3',4'-dihydrodurmillone with alkaline hydrogen peroxide.

Formation of 6-carbomethoxy-5-hydroxy-8-methoxy-2,2-dimethylchroman (VII) *and methyl piperonylate.* Dihydrodurmillone (50 mg) was added to a 5% soln (5 ml) of KOH in aqueous EtOH (80% EtOH) and the stirred soln was warmed at 42·5–45° during 1 hr and at 48–52° for a further hr. During the 2 hr period, sufficient 30% H_2O_2 was added at 15 min intervals to maintain a gentle evolution of O_2; the soln was initially deep yellow and then became colourless. The soln was then cooled, water (50 ml) was added, and it was shaken with ether. Carbon dioxide was then passed through the alkaline soln and the $CHCl_3$ extraction yielded a yellow oil (2 mg) which was not examined further. The bicarbonate soln was then

[49] F. P. Clark, *J. Am. Chem. Soc.* **53**, 313 (1931).
[50] K. Aghoramurthy, N. Narasimhachari and T. R. Seshadri, *Proc. Ind. Acad. Sci.* **33A**, 257 (1951).
[51] F. Wessely, L. Kornfeld and F. Lechner, *Ber. Dtsch. Chem. Ges.* **66B**, 685 (1933).
[52] W. Baker and F. M. Eastwood, *J. Chem. Soc.* 2897 (1929).
[53] A. Bolleter, K. Eiter and H. Schmid, *Helv. Chim. Acta* **34**, 186 (1951).

acidified (pH = 2) with 2N HCl and $CHCl_3$ extraction gave acidic material (25 mg) which was dissolved in MeOH (3 ml) and rapidly exposed to excess diazomethane in ethereal soln. After 20 sec, glacial AcOH was added and the mixture was evaporated under diminished press. The residue (30 mg) was separated by TLC (benzene) and two main bands (1 and 2) were detected by examination under UV light and spraying with ethanolic ferric chloride.

Band 1 gave an intense blue fluorescence under UV light but no coloration with ferric chloride. Extraction gave a white solid (14 mg) and crystallization from ether–light petroleum gave methyl piperonylate (10 mg) as white needles, m.p. 50°, identical (m.p. and IR spectrum) with an authentic specimen. Alkaline hydrolysis by heating (1 hr) of the methyl piperonylate (10 mg) with 10% KOH aq (0·2 ml) in 95% EtOH (1 ml) gave piperonylic acid (10 mg), m.p. 232°, by crystallization from ether–light petroleum followed by crystallization from hot water.

Band 2 gave a blue coloration with ethanolic ferric chloride spray and extraction gave a crystalline solid (16 mg). This was recrystallized from ether light petroleum followed by n-hexane giving 6-carbomethoxy-5-hydroxy-8-methoxy-2,2-dimethylchroman, m.p. 112–113·5°, identical (mixed m.p. and IR spectrum) with an authentic specimen (lit. m.p. 111·5–113°).[26,28]

Oxidation of milldurone with alkaline hydrogen peroxide

Formation of methyl 2-hydroxy-4,5-dimethoxybenzoate and methyl 6-methoxypiperonylate. Oxidative degradation of milldurone (50 mg) as in the preceding experiment gave methyl 2-hydroxy-4,5-dimethoxybenzoate (10 mg) as white needles, m.p. 95–96°, (lit. m.p. 95°)[30] from ether light petroleum and methyl 6-methoxypiperonylate (15 mg) as prisms, m.p. 75° (lit. m.p. 73–75°),[54] from ether light petroleum. These methyl esters were shown to be identical (mixed m.p. and IR spectra) with authentic samples.[30,54]

Acetylation of compound $C_{21}H_{22}O_4$. The compound $C_{21}H_{22}O_4$ (25 mg) with acetic anhydride (1 ml) and pyridine (2 drops) was kept at room temp (12 hr). Addition of water followed by extraction with ether gave a mixture which was separated (TLC) to give a crystalline *mono-acetate*, (6·5 mg), m.p. 105–107°, and an oil which was presumed to be a di-acetate (ν_{max} in $CHCl_3$, 1760, 1655 and 1640 cm^{-1}). The mono-acetate [Found: M (high resolution mass spectrum) 380·16055. $C_{23}H_{24}O_5$ requires M: 380·16236] showed λ_{max} (in EtOH) 233 mμ (ε 23,500), 342 mμ (ε 33,800) and ν_{max} (in $CHCl_3$), 1765 and 1640 cm^{-1}. The mono-acetate gave a dark brown coloration with ethanolic ferric chloride whereas the presumed di-acetate gave no coloration.

6a,12a-Dehydrodihydromillettone (XVI). Millettone (500 mg) was hydrogenated (platinic oxide catalyst, 100 mg) in EtOH (100 ml) at room temp and atm press for 2·5 hr. The product was purified by TLC (benzene–$CHCl_3$, 1:1) and although it could not be crystallized it was clearly dihydromillettone (350 mg), λ_{max} (in EtOH), 235 mμ (ε 12,900), 291 mμ (ε 17,400), ν_{max} (in $CHCl_3$) 1675, 1040, 940 cm^{-1}.

A soln of I_2 (215 mg) in EtOH (3 ml) was added during 1 hr to a soln of dihydromillettone (250 mg) and anhyd AcOK (650 mg) in boiling EtOH (15 ml). After boiling for a further period (1 hr), the mixture was then cooled and the light yellow ppt (147 mg) collected. 50% H_2SO_4 aq (0·17 ml) was added to the filtrate and after warming concentration gave a further quantity of product (89 mg). Recrystallization from EtOH–$CHCl_3$ gave *6a,12a-dehydrodihydromillettone* (83%) as pale yellow needles, m.p. 234°. (Found: C, 69·55; H, 5·08. $C_{22}H_{18}O_6$ requires: C, 69·84; H, 4·80%.) λ_{max} (in EtOH) 234 mμ (ε 25,000), 278 mμ (ε 21,000), 311 mμ (ε 17,000) ν_{max} (in $CHCl_3$) 1645 cm^{-1}.

6a,12a-Dehydromillettone (XV). Dehydrogenation of millettone (80 mg) as in the preceding experiment gave *6a,12a-dehydromillettone* (51 mg, 64%) as pale yellow needles, m.p. 278°, from EtOH–$CHCl_3$; recrystallization gave m.p. 357°. (Found: C, 69·87; H, 4·37. $C_{22}H_{16}O_6$ requires: C, 70·13; H, 4·29%.) λ_{max} (in EtOH) 211 mμ (ε 26,800), 235 mμ (ε 31,900), 260 mμ (ε 32,500), 319 mμ (ε 16,000). ν_{max} (in $CHCl_3$) 1645 cm^{-1}.

Oxidation of 6a,12a-dehydrodihydromillettone (XVI) *with alkaline hydrogen peroxide*

Formation of dihydro-β-tubaic acid methyl ether (XVIII). 30% H_2O_2 was added dropwise at a rate sufficient to maintain a gentle evolution of O_2 to a soln of 6a,12a-dehydrodihydromillettone (70 mg) in 5% ethanolic KOH aq (10 ml, 80% EtOH) during 1 hr at 45° and a further 2 hr at 60°. The mixture was then cooled, diluted with water (50 ml), shaken with ether, acidified, and extracted with ether giving a yellow oil (40 mg). Esterification (20 sec) with excess ethereal diazomethane gave a mixture of esters which was separated by TLC (benzene). The major fraction (20 mg), giving a blue coloration with ethanolic ferric chloride, was

[54] R. T. Arnold and N. Bortnick, *J. Am. Chem. Soc.* **67**, 1797 (1945).

methylated by boiling (6 hr) in anhyd acetone (20 ml) with MeI (2 ml) and anhyd K_2CO_3 (100 mg). This gave an oil which was heated under reflux (1 hr) with MeOH (2 ml) and KOH aq (20%; 2 ml). Acidification and extraction with AcOEt gave dihydro-β-tubaic acid methyl ether (17 mg), m.p. 110–111°, (lit. m.p. 110–111°)[55] after recrystallization from ether–light petroleum, which was identical (mixed m.p. and IR spectrum) with a sample previously obtained by the degradation of mundulone.[55]

Dehydration of (−)-millettosin (XXI)

Formation of 6a,12a-dehydromillettone (XV). (−)-Millettosin (5 mg) was heated under reflux with methanolic H_2SO_4 (10%; 1 ml) for 30 min, cooled, diluted with water (25 ml), and extracted with $CHCl_3$. This yielded a yellow oil which was crystallized from EtOH–$CHCl_3$ giving 6a,12a-dehydromillettone (3 mg), m.p. 355°, which was identical (mixed m.p., UV, and IR spectra) with an authentic sample (see above).

Oxidation of millettone to give (±)-millettosin (see XXI)

N NaOH (0·75 ml) was added to a soln of millettone (150 mg) in EtOH (15 ml) and the mixture was aerated at room temp for 24 hr. After standing at 0° for a further 24 hr, the ppt (101 mg) was collected and separated by thick layer chromatography (benzene) which yielded recovered millettone (65 mg) and (±)-millettosin (25 mg), m.p. 247–248°. [Found: M (mass spectrum), 394. $C_{23}H_{18}O_7$ requires: M, 394]. λ_{max} (in EtOH) 215 mμ (ε 15,900), 232 mμ (ε 20,800), 251 mμ (ε 21,100), 273 mμ (ε 24,600), 308 mμ (ε 11,000), 321 mμ infl. (ε 9000). ν_{max} (in $CHCl_3$) 3508, 1674, 1639, 1590 cm^{-1}; this spectrum was superimposable upon the IR spectrum of (−)-millettosin.

Dehydration of (±)-tephrosin (XI) *and (−)-tephrosin* (XXII)

Formation of 6a,12a-dehydrodeguelin (XII). (±)-Tephrosin (17 mg) was heated under reflux for 40 min with 10% methanolic H_2SO_4 (1·5 ml). After cooling the ppt (14 mg) was collected and crystallization from MeOH gave 6a,12a-dehydrodeguelin, m.p. 230–231°, identical (mixed m.p., UV, and IR spectra) with an authentic specimen[48] (lit. m.p. 233°).[49]

Dehydration of (−)-tephrosin (30 mg) similarly yielded 6a,12a-dehydrodeguelin (23 mg).

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The mass spectra were kindly determined by Dr C. P. Falshaw using an A.E.I. MS-9 mass spectrometer and we thank him for his advice regarding their interpretation.

[55] B. F. Burrows, N. Finch, W. D. Ollis and I. O. Sutherland, *Proc. Chem. Soc.* 150 (1959); J. Nickl, *Chem. Ber.* **92**, 1989 (1959).