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NMR INVESTIGATIONS OF ROTENOIDS

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Abstract—Extensive NMR studies of major rotenoids have (1) verified the *cis* B/C ring fusion of rotenone; (2) confirmed the structure of the reduction-dehydration product of rotenone; (3) provided considerable evidence regarding the preferred conformations of rotenoids; (4) revealed an array of long-range couplings; and (5) pointed up analytically useful solvent effects. Incidentally, these studies also allowed assignment of NMR signals for essentially all protons of the major rotenoids in deuterochloroform.

The data reported in this paper have been accumulated in the course of work at the Northern Laboratory on rotenoids† from *Tephrosia vogelii* Hook. f., a potential domestic crop source of thse natural insecticides.[1a,b] For instance, to identify the derivative employed in a GLC method for rotenoids, we described in a previous publication the preparation of 12-deoxy-$\Delta^{12(12a)}$-dehydrorotenone (**6**, Fig 1) by sodium borohydride reduction of the rotenone (**1a**) CO group in refluxing isopropanol followed by acid-catalyzed dehydration. Conceivably a hydride shift could have produced the isomeric compound, 12-deoxy-$\Delta^{6a(12a)}$-dehydrorotenone instead. NMR resolved this question by verifying the structure **6**.

Before our GLC[2] and TLC[3] analytical procedures were developed to analyze the structural isomers rotenone and deguelin (**2a**), only rotenone alone[4] or the total amount of rotenone, deguelin, and several minor interfering compounds[5] could be determined in rotenoid-containing plant extracts. In NMR spectra of crude extracts the E-ring Me lines of rotenone and deguelin are in a region with many signals from other substances. However, a slight separation of the OMe proton bands of rotenone from those of deguelin in a deuterobenzene (C_6D_6) spectrum provides a qualitative and semiquantitative means for detecting and distinguishing the two isomers.

Extensive decoupling experiments, together with investigations of solvent effects and long-range couplings (i.e. between protons separated by more than three bonds), allowed us to assign NMR signals to nearly all protons of the major rotenoids. The resulting data provided information relevant to several additional structural and conformational points, notably verification of the *cis* B/C ring fusion.

In the Discussion, NMR data are from spectra of the compounds in $CDCl_3$ unless otherwise specified.

DISCUSSION

Confirmation of cis *B/C ring fusion.* Evidence[6] for the stereochemistry at C-12a in natural rotenoids is based on relation to the (*S*)-configuration[7] at C-6a. Previous NMR support for *cis* B/C ring fusion was the chemical shifts of H-1 for a series of rotenoids[8] and $J_{6a,12a}$ for the 12-keto-derivative[9] of **8** in $CDCl_3$. For rotenone (**1a**) itself the 6a proton pattern is obscured and the 12a proton doublet falls under the OMe signal. However in deuteropyridine, the 12a proton doublet is clearly visible since H-12a is deshielded whereas the A-ring OMe's are shielded relative to the $CDCl_3$ spectrum (Table 1). Because the doublet disappears upon addition of deuterium oxide, a base-catalyzed H-D exchange is indicated and the identity of the doublet is verified. The lack of change in the signal upon addition of water instead of deuterium oxide to the deuteropyridine solution shows that no reaction is occurring other than the H-D exchange (e.g., rearrangement). The rotenone 6a, 12a coupling constant of 4·0 Hz (Table 2) observed in deuteropyridine was verified in C_6D_6/trifluoroacetic acid-*d* (10:1 v/v). To rule out epimerization at C-12a, solvent was removed from these solutions, and the rotenone samples recovered from them were examined by NMR in $CDCl_3$. No differences were detectable between these repeat spectra and the original one in $CDCl_3$ summarized in Tables 1 and 2. In $CDCl_3$ a 4·1 Hz $J_{6a,12a}$ coupling constant is apparent for elliptone (**9**) in the easily observed H-12a doublet; *ca* 4·0 Hz for $J_{6a,12a}$ in deguelin (**2a**) and α-toxicarol (**2c**) may be determined from the H-6a multiplet. [The H-6a pattern of sumatrol (**1c**) merges with the H-7″ singlet.‡] The coupling constant of 4·0 Hz is in

†The term rotenoid as used here includes rotenone and other compounds containing the ring structure of compound **8** (Fig 1).

‡A double prime (″) indicates a proton that has its pattern at higher field than its geminal proton.

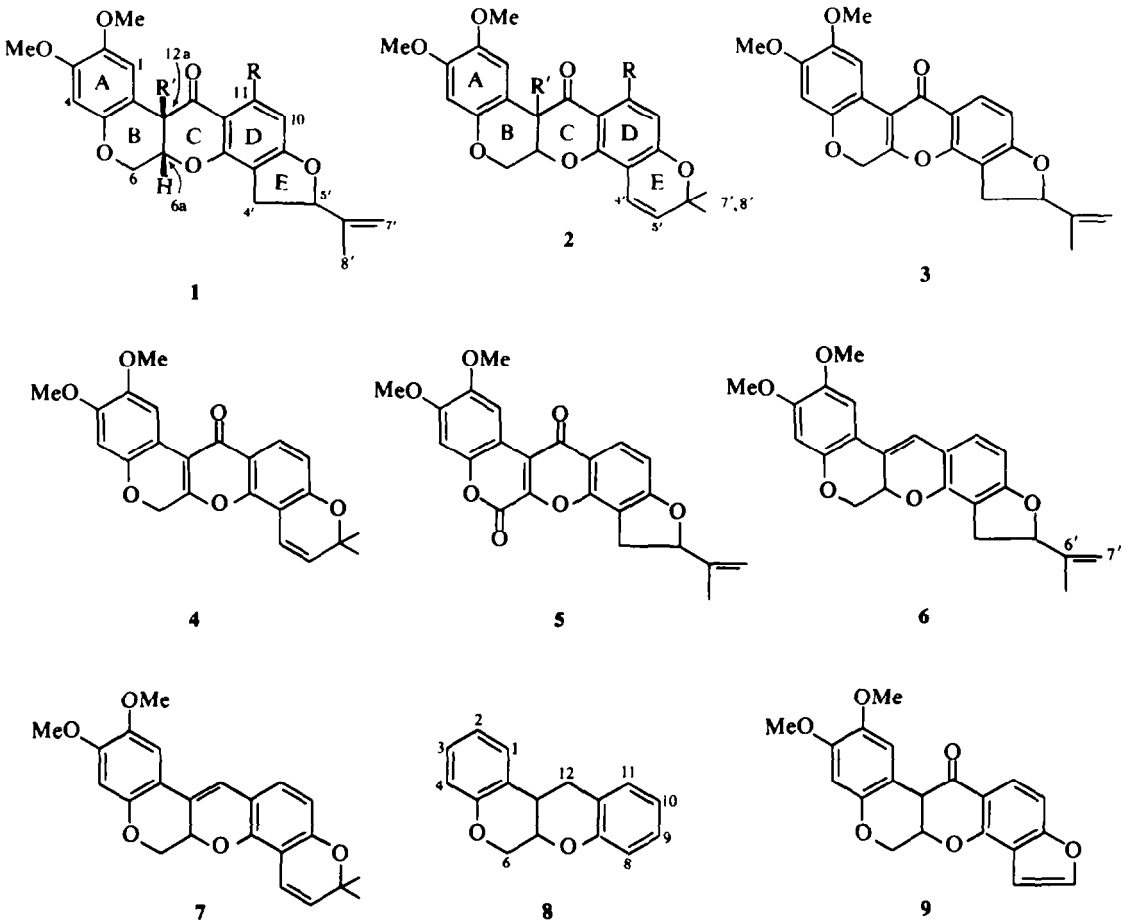

Fig 1. Rotenone (1a), deguelin (2a), tephrosin (2b), α-toxicarol (2c), sumatrol (1c), 6a,12a-dehydrorotenone (3), 6a,12a-dehydrodeguelin (4), rotenonone (5), 12-deoxy-$\Delta^{12(12a)}$-dehydrorotenone (6), 12-deoxy-$\Delta^{12(12a)}$-dehydrodeguelin (7), rotenoid ring system (9), elliptone (9).

reasonable agreement with the constant predicted by the Karplus equation[10] using parameters of Fraser *et al.*[11] for a 6a,12a-dihedral angle of 40° as measured from a Dreiding model. The constant definitely is not large enough to indicate a *trans* ring fusion. Thus the *cis* B/C ring fusion of rotenone, α-toxicarol, elliptone, and deguelin is confirmed by $J_{6a,12a}$. Based on $J_{6,6a}$ and $J_{6',6a}$ (see *Rotenoid conformation* Section) tephrosin (2b) likewise has a *cis* B/C ring fusion.

12-Deoxy-$\Delta^{12(12a)}$-dehydrorotenoids. A prime structural point resolved by NMR was the position of the double bond in the C-ring of the reduction-dehydration product of rotenone (1a), believed to be 12-deoxy-$\Delta^{12(12a)}$-dehydrorotenone (6). The presence of the H-6a multiplet (Table 1) offers proof that this double bond is at position 12,12a rather than 6a,12a. Furthermore, the 85° angle θ, measured from a Dreiding model (Fig 2A), is apparent from the allylic $J_{6a,12}$ of *ca* 2·4 Hz (Table 2). Allylic coupling reaches its maximum *ca* 3 Hz when this angle is 90°.[12] The absence of the CO is reflected in the lack of deshielding of H-11, which results in a 0·99 ppm upfield shift relative to rotenone.

During the reduction-dehydration, epimerization could have occurred at either C-6a or C-5'. Evidence of two isomers in the reduction-dehydration product of rotenone is found in the two overlapping four-line multiplets remaining in the H-6a pattern upon irradiation of H-12 (C_6D_6). There should, of course, be only one four-line pattern due to $J_{6,6a}$ and $J_{6',6a}$. The two H-4' protons ($CDCl_3$) also show twice the expected number of lines. The most

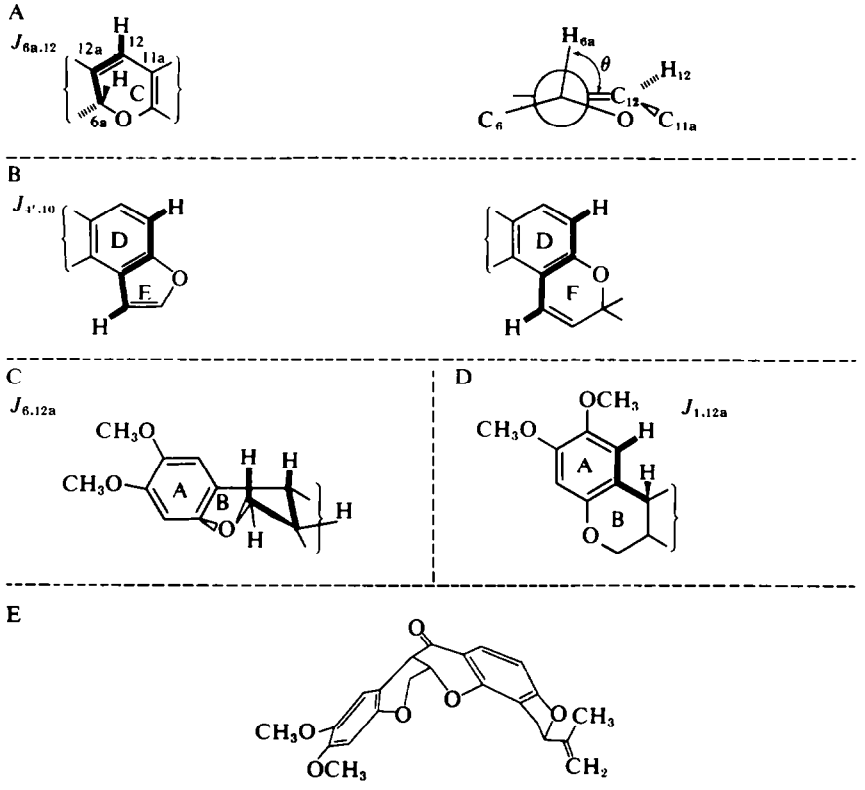

Fig 2. Long-range couplings in NMR spectra of rotenoids (A-D) and predominant conformation of rotenone (E).

conclusive evidence is the barely visible two-line C-8′ Me pattern ($CDCl_3$), which in C_6D_6 is resolved into two broadened Me singlets separated by 0·04 ppm. In C_6D_6 the major component has H-6a and H-8′ resonances centered at τ 4·77 and 8·40, respectively, whereas the minor component has corresponding resonances at τ 4·70 and 8·44. In $CDCl_3$ the patterns of the major component's two C-4′ protons are centered at τ 6·75 and 7·06, and those of the minor component are centered at τ 6·70 and 7·10. Small differences in resonance could be present but obscured in the two broad H-7′ and in the H-5′ signals. The peak height of the high-field CH_3-8′ singlet (C_6D_6) ranged from 36–77% of that of the low-field one depending upon the reaction procedure.

The 12-deoxy-$\Delta^{12(12a)}$-dehydro-6′,7′-dihydrorotenone also proved to be a mixture of isomers, which is not surprising, since it was prepared[13] from 6′,7′-dihydrorotenone by the same reduction and dehydration procedure used to prepare 12-deoxy-$\Delta^{12(12a)}$-dehydrorotenone from rotenone. The epimerization was most evident in the C-7′ and C-8′ Me patterns, which overlapped but which were resolved in C_6D_6 into two major doublets ($J_{6',7'} = J_{6',8'} = 6{\cdot}6$ Hz) at τ 9·06 and 9·22 and two minor doublets ($J_{6',7'} = J_{6',8'} = 6{\cdot}6$ Hz) at τ 9·09 and 9·25. As in 12-deoxy-$\Delta^{12(12a)}$-dehydrorotenone, the Me signals of the minor isomer are at higher field than those of the major isomer.

Rotenoid conformation. Limited tests of the effects of molecular permutations on biological activity of rotenoids and related compounds[14a,b,c] have given results that defy simple structure-activity generalizations. While certain minor chemical changes completely destroyed the physiological property measured, other modifications seemingly far more profound did not. Since conformation is an important factor in determining biochemical effects of organic compounds, we examined our NMR data for insight into molecular shape.

The magnitude of the coupling between H-6a and the geminal H-6 protons reveals the predominant conformations of the B- and C-rings. As has been reported[9] for the 12-keto-derivative of **8** in $CDCl_3$, we found that the 6*ax* proton of rotenone (**1a**), distinguished from H-6*eq* by smaller coupling with H-6a (Table 2), resonates at higher field (Table 1) than the geminal H-6*eq*. The $J_{6ax,6a}$ *ca* 1·2 Hz and $J_{6eq,6a} = 3{\cdot}1$ Hz indicate 6*ax*,6a (6″,6a) and 6*eq*,6a dihedral angles[11] that are in good agreement with values of 65° and 55°, respectively, read from a Dreiding model (Fig 3A). A *cis* B/C ring fusion, together with any of the other three possible com-

Table 1. Chemical shifts (τ) of rotenoid protons[a]

Compound	Solvent	H-1s	H-4s	H-6	H-6a	H-12a or H-12$^+$	Me-2, Me-3	H-10d	H-11d	H-4′	H-5′	H-7′b	Me-8′[b]
Rotenone (1a)	$CDCl_3$	3·23	3·55	5·41q[c] 5·85bd[c]	5·1	6·2	6·21[c] 6·25[c]	3·50	2·16	6·68q[c] 7·07q[c]	4·78t[c]	4·94[c] 5·1	8·23b
	C_5D_5N	2·8	3·31	5·22 5·73bd	4·9	5·86bd	6·37 6·39	3·42	1·91	6·9 7·1	4·8	4·9 5·1	8·33b
	C_6D_6	2·9	3·50	5·76q 6·5	5·94t	6·4	6·61[c] 6·74[c]	3·62	1·89	7·31d 7·31d	5·29	5·09 5·3	8·54b
	C_6D_6 + TFA-*d*	3·18	3·64	5·83q[c] 6·59d[c]	6·03t[c]	6·45bd[c]	6·61[c] 6·81[c]	3·71	2·13	7·36d[c] 7·36d[c]	5·32[c]	5·15[c] 5·3	8·57b
Sumatrol (1c)	$CDCl_3$	3·14b	3·56	5·44q[c] 5·87bd[c]	5·2m	6·2	6·20[c] 6·23[c]	4·00[d]	—	6·77q[c] 7·17q[c]	4·83t[c]	4·96[c] 5·10[c]	8·26b
	C_6D_6	2·9	3·53[e]	5·82q[c] 6·57[c]	6·08t[c]	6·6	6·63[c] 6·75[c]	3·77[d,e]	—	7·39d[c] 7·39d[c]	5·35t[c]	5·13[c] 5·35[c]	8·56b
12-Deoxy-$\Delta^{12(12a)}$-dehydrorotenone (6)-major isomer	$CDCl_3$	3·01	3·59	5·45q[e] 5·91t[e]	4·73m[f]	3·41$^+$d	6·13[e] 6·18[e]	3·61	3·15	6·75q[e] 7·06q[e]	4·84t[f]	4·92 5·10[e]	8·23b[e]
	C_6D_6	3·02	3·60	5·51q 5·85t	4·77m[f]	3·47$^+$d	6·50[e] 6·72[e]	3·47	3·22	7·0 7·1	5·07	4·94 5·24	8·40b
12-Deoxy-$\Delta^{12(12a)}$-dehydro-6′,7′-dihydrorotenone-major isomer	$CDCl_3$	3·02[e]	3·61[e]	5·45q[e] 5·90t[e]	4·73m[e]	3·42$^+$d[e]	6·12[e] 6·17[e]	3·67[e]	3·19q[e]	6·9 7·2	5·5	—	8·96d 9·03d
	C_6D_6	3·02	3·60	5·48q 5·84t	4·73m	3·5$^+$	6·51[e] 6·72[e]	3·48	3·22	7·1 7·3	5·8	—	9·06d 9·22d
6a,12a-Dehydrorotenone (3)[g]	$CDCl_3$	1·56[e]	3·48[e]	5·03s 5·03s	—	—	6·06[e] 6·16[e]	3·11[e]	1·89[e]	6·48[e] 6·85[e]	4·61t	4·88 5·03	8·20b
Rotenonone (5)[g]	$CDCl_3$	1·05[e]	3·15[e]	—	—	—	5·99[e] 6·06[e]	3·04[e]	1·86[e]	6·31q 6·67q	4·53t	4·85 5·00	8·18b
Deguelin (2a)	$CDCl_3$	3·21b[e]	3·57[e]	5·38q[e] 5·84bd[e]	5·10m[e]	6·2	6·21[e] 6·25[e]	3·57b[e]	2·27[e]	3·37bd[e]	4·46d[e]	—	8·56[d] 8·63[d]
	C_6D_6	2·9	3·58[e]	5·78q[e] 6·52[e]	6·00m[e]	6·48	6·63[e] 6·78[e]	3·57b[e]	1·99[e]	3·36bd[e]	4·91d[h]	—	8·82 8·97
α-Toxicarol (2c)	$CDCl_3$	3·14b[e]	3·56[e]	5·40q[e] 5·86bd[e]	5·16m[e]	6·2	6·20[e] 6·23[e]	4·06b[e,d]	—	3·46bd[e]	4·56d[e]	—	8·57 8·64
	C_6D_6	2·9	3·59	5·86q 6·60	6·2m	6·7	6·64[e] 6·78[e]	3·73b[d]	—	3·43bd	4·96d	—	8·83 8·97
Tephrosin (2b)	$CDCl_3$	3·45[e]	3·54[e]	5·5 5·5	5·5	—	6·20[e] 6·28[e]	3·56b[e]	2·30[e]	3·42bd[e]	4·47d[e]	—	8·56 8·62
	C_6D_6	3·14	3·61	5·6 5·7	5·92[e]	—	6·71[e] 6·85[e]	3·61b	2·11	3·43bd	4·95d	—	8·85 9·03

12-Deoxy-$\Delta^{12(12a)}$-dehydrodeguelin (7)	$CDCl_3$	3·02[c]	3·60[c]	5·42q[c] 5·87t[c]	4·73m[c]	3·46'd[c]	6·12[c] 6·17[c]	3·64b[c]	3·19[c]	3·40bd[c]	4·42d[c]	—	8·57 8·60
	C_6D_6	3·07	3·63	5·58q 5·91t	4·86m	3·57+d	6·53[c] 6·74[c]	3·41b	3·26	3·23bd	4·67d	—	8·67 8·72
6a,12a-dehydrodeguelin (4)[g]	$CDCl_3$	1·55	3·46	5·00s 5·00s	—	—	6·06[c] 6·15[c]	3·15b	1·97	3·25bd	4·29d	—	8·52
Elliptone (9)	$CDCl_3$	3·23b[c]	3·55c	5·29q[c] 5·77bd[c]	4·93m[c]	6·06bd[c]	6·22[c] 6·26[c]	2·87b[c]	2·11[c]	3·09q[c]	2·46d[c]	—	—
	C_6D_6	2·9	3·57[c]	5·72q[c] 6·51[c]	5·88m[c]	6·42[c]	6·62[c] 6·78[c]	3·18b[c]	1·93[c]	3·44q[c]	3·15d[c]	—	—

[a]Abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broadened singlet, bd = broadened doublet. Values of two significant figures indicate approximations from multiplets, overlapping signals, or broad signals. The scale was 2 Hz/mm (1000 Hz sweep width) unless otherwise noted.

[b]7'-Me and 8'-Me for compounds having a deguelin E-ring or for 6',7'-dihydrorotenone.

[c]Scale 0·5 Hz/mm, sweep rate 1 Hz/sec.

[d]This signal is a singlet.

[e]Scale 1 Hz/mm, sweep rate 2 Hz/sec.

[f]Ascertained through decoupling.

[g]Insufficiently soluble in C_6D_6 to obtain a spectrum.

[h]Scale 1 Hz/mm, sweep rate 1 Hz/sec.

Table 2. Coupling constants in rotenoids[a]

Compound	$J_{6,6a}$	$J_{6'',6a}$	$J_{6,6''}$	$J_{6a,12a}$	$J_{6a,12}$	$J_{10,11}$	$J_{4',4''}$	$J_{4',5'}$	$J_{4'',5'}$	$J_{4',10}$	$J_{1,12a}$
Rotenone (**1a**)	3·1[b]	*ca* 1·2[c]	12·1[b]	4·0[d]	—	8·6	15·9[b]	9·5[b]	8·2[b]	N.O.[e]	N.O.
Sumatrol (**1c**)	3·1[b]		11·8[b]		—	—	15·2[b]	9·5[b]	7·8[b]	N.O.	*ca* 0·6[c]
12-Deoxy-$\Delta^{12(12a)}$-dehydrorotenone (**6**)	5·1[f]	11·0[f]	9·9[f]	—	2·4	8·0	15·6[f]	9·4[f]	8·2[f]	N.O.	—
12-Deoxy-$\Delta^{12(12a)}$-dehydro-6′,7′-dihydrorotenone	5·5[b]	10·9[b]	10·0[b]	—	*ca* 2·0[b]	7·9[b]				N.O.	—
6a,12a-Dehydrorotenone (**3**)	—	—		—	—	8·6[b]	16·0[b]	9·6[b]	8·2[b]	N.O.	—
Rotenonone (**5**)	—	—	—	—	—	8·6[b]	16·5	9·2	7·8	N.O.	—
Deguelin (**2a**)	3·2[b]	*ca* 1·0[b]	11·8[b]	*ca* 4·0[b]	—	8·7[b]	—	10·1[b]	—	*ca* 0·6[b,c]	*ca* 0·8[b,c]
α-Toxicarol (**2c**)	3·1[b]	*ca* 1·3[b]	12·1[b]	*ca* 4·0[b]	—	—	—	10·0[b]	—	*ca* 0·7[b,c]	*ca* 0·7[b,c]
Tephrosin (**2b**)	*ca* 1·2[b,g]	*ca* 2·1[b,g]	*ca* 12·2[b,g]	—	—	8·6[b]	—	10·0[b]	—	*ca* 0·7[b,c]	—
12-Deoxy-$\Delta^{12(12a)}$-dehydrodeguelin (**7**)	5·4[b]	10·8[b]	10·0[b]	—	2·4[b]	8·2[b]	—	10·0[b]	—	*ca* 1·0[b,c]	—
6a,12a-Dehydrodeguelin (**4**)	—	—	0	—	—	8·8[b]	—	10·2	—	*ca* 0·5[b,c]	—
Elliptone (**9**)	3·4[b]	*ca* 1·0[b]	11·8[b]	4·1[b]	—	8·6[b]	—	2·0[b]	—	1·0[b]	*ca* 0·9[b,c]

[a]H-4″ and H-6″ are protons that resonate at higher field than their identically numbered counterparts. Solvent was $CDCl_3$ and scale was 2 Hz/mm (1000 Hz sweep width) unless otherwise noted.

[b]Scale 0·5 Hz/mm.

[c]Measured by width at half height.

[d]Determined in C_5D_5N and in C_6D_6-TFA (10 : 1).

[e]Not observed.

[f]Scale 1 Hz/mm.

[g]Determined in C_6D_6.

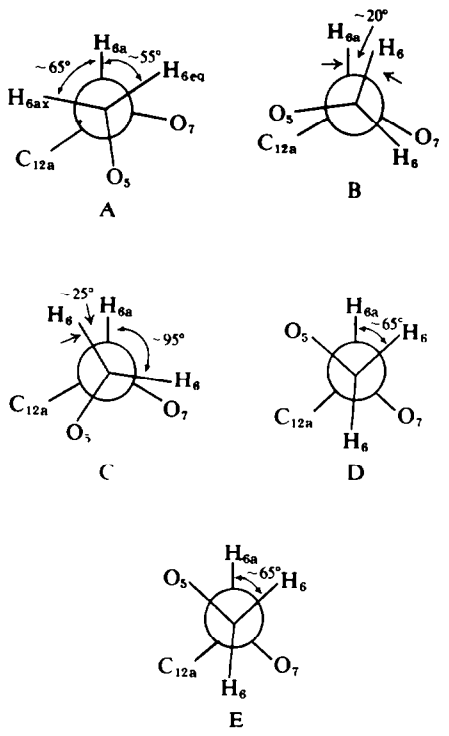

Fig 3. Newman projection along C_6-C_{6a} bond reflecting conformational alternatives in B- and C-rings. (A) and (B) two B-ring alternatives of rotenone with the C-ring fixed in the preferred conformation; (C) and (D) two B-ring alternatives of rotenone with the C-ring in its alternate conformation; (E) 12-deoxy-$\Delta^{12(12a)}$-dehydrorotenone.

binations of B- and C-ring conformations (Fig 3B-D), would give predicted couplings between H-6a and the two C-6 protons significantly different from those observed. If instead the B/C ring juncture were *trans*, one coupling constant, $J_{6ax,6a}$, would be large (10-14 Hz). Similarly, the two small couplings $J_{6,6a}$ *ca* 1·2 and $J_{6',6a}$ *ca* 2·1 Hz (C_6D_6) for tephrosin (**2b**) verify earlier predictions, based on H-1 chemical shifts[8] and H-bonding,[15] that the B/C ring juncture of tephrosin is *cis* as in rotenone. (The signals of the C-6a and the two C-6 protons are not resolved in $CDCl_3$). The $J_{6,6a}$ (i.e. $J_{6eq,6a}$) values for elliptone (**9**), deguelin (**2a**), sumatrol (**1c**), α-toxicarol (**2c**), and rotenone in both $CDCl_3$ (Table 2) and C_6D_6 are nearly equivalent. Therefore, the B- and C-ring conformations of these compounds are all identical and are the same in both solvents.

The C-1 proton in tephrosin is shielded 0·24 ppm in comparison with deguelin. This H-1 shielding could be the result of a slight conformational change from that of deguelin, as suggested by differences in $J_{6,6a}$ and in $J_{6',6a}$.

Introduction of a 12,12a double bond has a marked conformational effect. The *trans*-diaxial relationship (Fig 3E) between H-6a and one of the C-6 protons in 12-deoxy-$\Delta^{12(12a)}$-dehydrorotenone (**6**) is reflected in the large coupling constant ($J = 11·0$ Hz). Smaller coupling ($J = 5·1$ Hz) is observed between H-6a and the equatorial C-6 proton. In contrast the C-6a proton of rotenone nearly bisects the angle between the two C-6 protons (axial and equatorial) and, consequently, has small couplings with both. Comparison of $J_{6,6a}$ values and of $J_{6',6a}$ values indicates that the B-ring stereochemistry of 12-deoxy-$\Delta^{12(12a)}$-dehydro-6′, 7′-dihydrorotenone and 12-deoxy-$\Delta^{12(12a)}$-dehydrodeguelin (**7**) is the same as that of 12-deoxy-$\Delta^{12(12a)}$-dehydrorotenone.

The near planarity of the C/D-ring system is emphasized by the H-bonding in sumatrol and α-toxicarol between the C-11 OH and the C-ring CO oxygen. This H-bonding has been documented elsewhere by TLC migration[16] compared to rotenone, deguelin, and tephrosin. In the IR spectra of these hydroxylated compounds a broad OH absorption is seen at 3500 cm^{-1} for tephrosin but is so broad in α-toxicarol and sumatrol (5% chloroform) that it is not observed. Also, the CO band of tephrosin is at the same wavelength as it is in deguelin (1670 cm^{-1}) while the CO of α-toxicarol absorbs at 1645 and that of sumatrol at 1650 (relative to 1670 cm^{-1} in rotenone). These frequency decreases are approaching the magnitude seen in flavanone[17] upon introduction of a C-5 OH moiety that H-bonds to the CO. It is therefore not surprising that sumatrol and α-toxicarol have their OH protons deshielded down to chemical shifts close to that of the chelated 5-OH group in a number of flavones and flavonones at comparable concentrations in $CDCl_3$.[18] The OH protons of α-toxicarol, sumatrol, and tephrosin appear at τ −2·2, −2·4, and 5·6 in $CDCl_3$ and at τ −2·8, −3·1, and 5·5 in C_6D_6.

The E-ring of rotenone appears to have the envelope conformation.[19] Coupling constants of 8·2 and 9·5 Hz observed between C-4′ and C-5′ protons are in fair agreement with predictions from 150 and 30° angles measured from a Dreiding model wherein the E-ring has the envelope conformation with the isopropenyl group equatorial and the 5′ proton α and axial.[6] The evidence is thus indicative of this conformation as the major one, with some small contribution from the 5′ proton in the equatorial position possible on a time-averaged basis. Since there is little difference in the two dihedral angles from the coupling constant 8·2 and the two from 9·5 Hz given by the Karplus relationship, unequivocal assignments of each four-line multiplet to a specific C-4′ proton are not possible.

Collectively, the NMR evidence indicates the predominant overall conformation shown in Fig 2E for rotenone.

Long-range couplings. The spin-spin couplings of approximately 1·0 Hz across five bonds observed in compounds containing the benzofuran

moiety[20a,b] are equivalent to the type of coupling seen (Table 2) between the C-4′ and C-10 protons (Figs 2B and 4A) in elliptone (9). Small couplings have been noted through the comparable zig-zag path in compounds containing the 1,2-benzopyran ring system present in the deguelin-type D- and E-rings.[21] Sternhell and associates[22a,b] have observed both the larger $J_{3,7}$ (corresponding to $J_{4',10}$ in elliptone) and smaller $J_{2,6}$ ($J_{5',11}$ in elliptone) in benzo[*b*]thiophens. However, only $J_{4',10} = 0{\cdot}7$ Hz was reported by Ollis *et al.*[23] for millettone, which has a deguelin-like structure where the A-ring OMe groups are replaced by a methylenedioxy group.

As expected[24] from the complete planarity of the D/E-ring system, the largest $J_{4',10}$ (1·0 Hz) observed for the rotenoids in our survey is exhibited by elliptone. The fine splitting evident even in the 1000 Hz sweep width spectrum of elliptone is resolved in the 250 Hz sweep width spectrum (Fig 4A) into two distinct four-line patterns. Thus the earlier assignments[25] of elliptone's H-11 and H-5′ patterns at lower field than those of H-10 and H-4′, respectively, are substantiated (Table 1).

Replacing the furan elliptone E-ring with the deguelin-type pyran E-ring shifts H-4′ slightly out of the plane in which C-4′, C-3′, C-2′, C-10, and H-10 lie. This less favorable relationship for long-range coupling is reflected in the approximately 0·7 Hz $J_{4',10}$ determined for tephrosin (2b) by measuring peak widths at half heights. Nevertheless, a 250 Hz sweep width spectrum of tephrosin in the τ 2·2 to 4·5 region (Fig 4B) allows the H-4′ and H-10 doublets to be distinguished from the H-5′ and H-11 doublets, respectively. Evidently due to the small differences between $J_{4',5'}$ and $J_{10,11}$, the assignments of chemical shifts of H-4′ and H-10 were previously reversed when a 60 MHz instrument was employed.[25] However, the greater accuracy of these J values from the 100 MHz spectrum along with the coupling between H-4′ and H-10 allows positive identification of the H-4′ doublet at τ 3·42 and of the H-10 doublet at τ 3·56. In the deguelin (2a) 250 Hz sweep width spectrum the H-4′ doublet lines are broadened and the H-10 doublet is partially split. The width at half height of the broadened H-10 and H-4′ doublets in the spectra of deguelin, 6a,12a-dehydrodeguelin (4), α-toxicarol (2c), and 12-deoxy-$\Delta^{12(12a)}$-dehydrodeguelin (7) indicate couplings (Table 2) of *ca* 0·5–1·0 Hz.

Investigations were made of $J_{5',8'}$ and $J_{6'',12a}$ ($J_{6ax,12a}$) (Fig 2C), the two possible couplings across four sigma bonds (i.e. ^4J). If present, $J_{5',8'}$ (and $J_{5',7'}$ in compounds having a 7′-Me group) was not readily apparent in any of the compounds. The second ^4J investigated, $J_{6'',12a}$, was readily discerned in the elliptone spectrum (0·5 Hz/mm) where the finely split 12a proton doublet is out from under the OMe proton resonances ($J_{6'',12a}$ *ca* 0·8 Hz). Among rotenone, deguelin, sumatrol (1c), and α-toxicarol—the other rotenoids in Table 2 which have H-12a—deguelin showed shoulders on the H-6″ doublet when decoupled from H-6a, whereas the H-6″ doublets of rotenone, sumatrol, and α-toxicarol still appeared broadened when H-6a was irradiated. No broadening of the H-6 four-line pattern was apparent. The proximity of H-6″ and H-12a resonances did not allow decoupling for verification. Supporting evidence for $J_{6'',12a}$ was found in the spectra of rotenone dissolved in either C_5D_5N or C_6D_6-TFA-*d*. In these spectra the H-12a doublet was broad compared to the H-10 and H-11

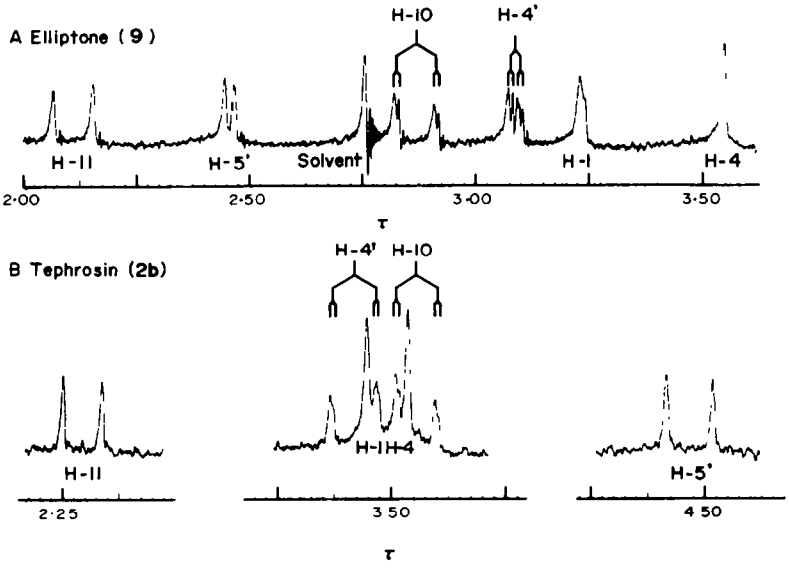

Fig 4. Portions of NMR spectra ($CDCl_3$, 0·5 Hz/mm) showing $J_{4',10}$

doublets. In general the homoallylic *para* $J_{1,4}$ was not investigated. However, in rotenonone (5), in which H-4 was irradiated, the H-1 singlet was unaffected.

The allylic coupling between H-1 and H-12a (Fig 2D and Table 2) has been reported for millettone,[23] and the corresponding coupling has been noted in compounds possessing similar ring systems.[26] This coupling was present in elliptone, sumatrol, deguelin, and α-toxicarol, but it was not observed in the other rotenoids. The $J_{1,12a}$ coupling allowed us to distinguish between H-1 and H-4 singlets, as is readily seen for elliptone in Fig 4A. The H-1 line was the singlet at lower field as expected because of CO deshielding. Other allylic couplings were reflected in broadened peaks or increased multiplicity, but none were measurable.

Solvent effects. Since formation of quite stable solvates is a distinguishing characteristic of rotenoids,[4,27a,b] chemical shifts in $CDCl_3$ were compared with those in C_6D_6 to glean evidence for preferred sites of interaction of C_6D_6 with a rotenoid molecule. This solvent interaction is readily apparent (Fig 5) from the increased chemical shift difference of the OMe proton signals of C_6D_6 spectra as compared with $CDCl_3$ spectra, resulting in a separation of these signals for rotenone (1a) from the corresponding ones for deguelin (2a). The spectrum of a 1:1 mixture of these two isomers shows that heights of OMe methyl peaks of rotenone are nearly equivalent to those for deguelin. An increase in the rotenone OMe lines without a corresponding increase in those of deguelin, due to an unknown compound (or compounds) in benzene or acetone extracts of *Tephrosia vogelii* leaflets, restricts the estimation of relative amounts of these two components from the OMe proton NMR signals to fairly clean samples. Since 6a,12a-dehydrorotenone (3), 6a,12a-dehydrodeguelin (4), and rotenonone (5) are all insufficiently soluble in C_6D_6 to give spectra, presumably they would not interfere in this rotenone-deguelin analysis. At this time we can offer no explanation for the small, but real, differences in chemical shifts for rotenone and deguelin A-ring Me signals, but during our efforts to find an explanation other solvent-rotenoid relationships were brought to light.

The separation of OMe proton sign of the $\Delta^{12(12a)}$-rotenoids in C_6D_6 is significantly greater than the other rotenoids. It is felt that this is due to donation of electron density by the C-3 OMe oxygen through the C-ring double bond to the now conjugated D- or E-ring which is favorable in C_6D_6 (and not in $CDCl_3$) owing to association with the solvent.

Judging from the large shielding of rotenone's H-6a in C_6D_6 relative to $CDCl_3$ (Table 1), the benzene molecule on a time-average basis lies predominantly over this proton. The preferred benzene orientation is apparently the same for elliptone (9) and deguelin, which have H-6a shielded 0·95 and 0·90 ppm, respectively, and for α-toxicarol (2c) and sumatrol (1c) (both *ca* 1 ppm). The C-2 OMe protons may also be affected by this C_6D_6 association. The paramagnetic shift of the H-1 singlet (0·2–0·3 ppm for rotenoids containing a CO group in the C-ring) in C_6D_6 relative to $CDCl_3$ is common for protons lying in the vicinity of a CO and on the oxygen side of a reference plane through the carbon of the CO and perpendicular to the direction of the C=O bond.[28] The significant deshielding of H-1

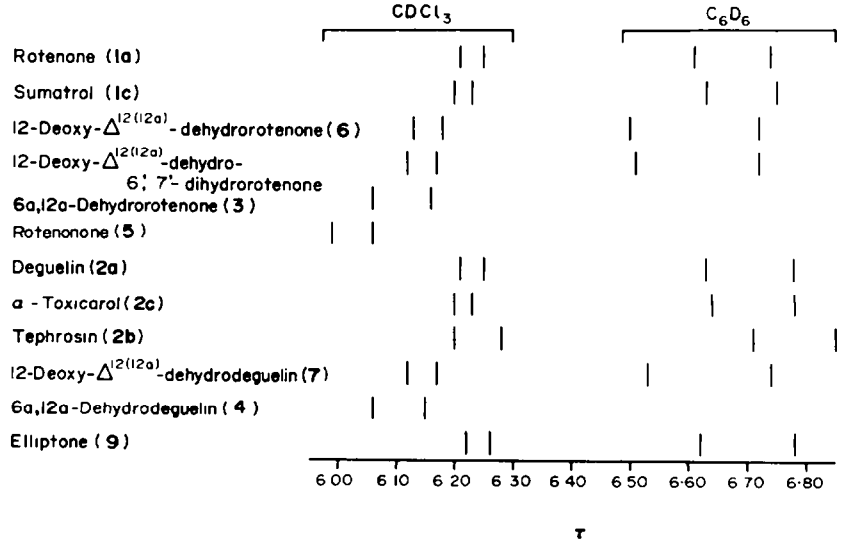

Fig. 5 Chemical shifts of A-ring OMe protons in $CDCl_3$ and (where solubility permitted) in C_6D_6(τ).

may well be carrying over to the C-2 OMe group and causing the somewhat greater difference in chemical shifts between the C-2 and C-3 rotenone OMe protons in C_6D_6 compared to the small difference in $CDCl_3$ (0·13 *vs* 0·04 ppm, Fig 5). Corresponding increases in separation of the OMe proton lines are seen in Fig 5 for the other major rotenoids which possess the C-ring CO. In addition to this differential effect on the two A-ring OMe groups, C_6D_6 also seems to have a second interaction with both of them. The considerable shielding of the OMe methyl protons, the negligible shift of the H-4 signal (−0·05 to +0·03 ppm) in all the compounds without a 12a OH, and the negligible shift of the H-1 signal (0 to 0·05 ppm) in the three compounds without a C-ring CO are readily apparent. These phenomena fit the proposed[29] orientation where benzene lies over an OMe group in a position to deshield slightly the aromatic proton *ortho* to the OMe.

EXPERIMENTAL

NMR spectra (TMS internal standard, chemical shifts in τ, J in Hz) were measured with a Varian* HA-100 spectrometer. NMR solns were 0·08 to 0·10M, except for rotenonone which was 0·03M. Larger coupling constants were read directly from the spectra as were chemical shifts. The chemical shift of a multiplet was taken to be the center of the pattern. Smaller coupling constants were often determined by measuring the widths at half height as noted in Table 2. The H-4 singlet was compared with the H-1 line for $J_{1,12a}$ determination. Likewise, constants for $J_{4',10}$ were obtained by comparison with either the H-11 or the H-5' doublet while E-ring Me singlets were compared to A-ring OMe methyl singlets. Scales and sweep times were 2 Hz/mm at 2 Hz/sec, 1 Hz/mm at 1 or 2 Hz/sec, and 0·5 Hz/mm at 1 Hz/sec. The first condition was always in effect unless otherwise noted.

IR (5% $CHCl_3$ solns, 0·1 mm NaCl cell) and UV (MeOH solns) were determined with a Perkin-Elmer Model 337 spectrophotometer and a Beckman Model DK-2A spectrophotometer, respectively.

Mass spectra were measured on a Nuclide 12–90 DF mass spectrometer with a direct probe inlet. The ionizing voltage was 70 eV. M.ps were determined by using a Kofler block.

IR data (ν_{max}) are given below in cm^{-1} while UV data (λ_{max}) are given in nm (log ϵ).

Rotenone (**1a**). Aldrich rotenone was crystallized from CCl_4 followed by recrystallization from EtOH to give transparent plates, m.p. 162·5–164·0°; ν_{max} 1670, 1615, 1520, 1465, 1360; λ_{max} 236 (4·16), 293 (4·25).

Sumatrol (**1c**). Transparent crystals had m.p. 174·5–177·5° (with dec); ν_{max} 1650, 1615, 1520, 1480, 1390, 1360; λ_{max} 297 (4·36).

Deguelin (**2a**). Acetone extracts of a rotenone-free line of *Tephrosia vogelii* leaflets were put through a multistep procedure, including column chromatography on Silica Gel G (Brinkmann, 0·05–0·20 mm), to obtain a deguelin-rich fraction. The eluant was either $CHCl_3$-Et_2O (95:5) or CH_2Cl_2-Me_2CO (98:2). Alkali treatment of this 930-mg fraction as described by Haller and LaForge,[30] followed by column chromatography using $CHCl_3$-Et_2O (95:5) as the eluant and crystallization from $CHCl_3$-MeOH followed by two recrystallizations from MeOH-Me_2CO and two from EtOH, produced a 16% yield of transparent rectangular plates of the racemic rotenoid, m.p. 155·5–158·0°; ν_{max} 1670, 1605, 1580, 1520, 1350; λ_{max} 238 (4·32), 250 (4·33), 269 (4·43), 298 (3·97).

Tephrosin (**2b**). White crystals had m.p. 197·5–199·5°; ν_{max} 3500, 1670, 1605, 1580, 1520, 1450, 1340; λ_{max} 238 (4·34), 250 (4·34), 271 (4·40), 299 (4·00).

6a,12a-*Dehydrorotenone* (**3**).† Oxidation of rotenone with MnO_2 (Beacon Chemical Industries, Inc.) was carried out as described by Highet and Wildman.[31] EtOH-free chloroform was used, and the mixture was refluxed for 24 hr. Chromatography of the product on a silicic acid (Mallinckrodt, 100 mesh) column with $CHCl_3$ as the eluant separated the faster moving 6a,12a-dehydrorotenone from rotenonone. Crystallization from $CHCl_3$-EtOH yielded pale yellow transparent needles of m.p. 223·5–227·5° (with dec); ν_{max} 1640, 1610, 1510, 1450, 1300; λ_{max} 238 (4·46), 278 (4·38), 309 (4·26); mass spectrum: 393 (27%), 392 (100, M^+), 377 (11), 345 (10). (Found: M^+ 392·126. Calc. for $C_{23}H_{20}O_6$: 392·126).

Rotenonone (**5**).† The reaction described under the 6a,12a-dehydrorotenone preparation produced material that eluted from the chromatographic column as an orange band. The rotenonone fraction was 10% of the original rotenone weight while the 6a,12a-dehydrorotenone fraction was 17%. Crystallization from $CHCl_3$-EtOH yielded transparent yellow needles of m.p. 298·0–300·5° (with dec); ν_{max} 1740, 1630, 1605, 1375; λ_{max} 261 (4·38), 268 (4·38), 297 (4·30), 342 (3·91); mass spectrum: 407 (26%), 406 (100, M^+), 392 (24), 391 (49). (Found: M^+ 406·102. Calc. for $C_{23}H_{18}O_7$: 406·105.)

α-*Toxicarol* (**2c**). Yellow plates of m.p. 217·0–222·5°; ν_{max} 1645, 1520, 1285; λ_{max} 228 (4·23), 235 (4·22), 273 (4·54), 296 (4·14).

6a,12a-*Dehydrodeguelin* (**4**). Transparent yellow needles of m.p. 224·5–230·5° (with dec); ν_{max} 1640, 1515, 1390; λ_{max} 220 (4·45), 235 (4·50), 260 (4·54), 312 (4·19).

12-*Deoxy*-$\Delta^{12(12a)}$-*dehydrorotenone* (**6**).[2] ν_{max} 1625, 1510, 1480, 1450; λ_{max} 219 (4·47), 255 (4·17), 264 (4·10), 362 (4·52), 380 (4·43).

12-*Deoxy*-$\Delta^{12(12a)}$-*dehydro*-6',7'-*dihydrorotenone*. Colorless crystals of m.p. 215·5–218·0°; ν_{max} 1620, 1510, 1480;

*The mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

†The behavior of 3 and 5 in the TLC analytical procedure[3] may be of interest. When dehydrorotenone (10 μg), rotenonone (11 μg), and rotenone (11 μg) were applied to a silver nitrate-impregnated TLC plate, respective peak weights from the densitometer recordings after plate development were 100, 5, and 161 mg. Rotenonone appeared as a gold spot after visualization with nitric acid vapors followed by ammonia vapors, whereas 6a,12a-dehydrorotenone had the same color as rotenone. Doubling the level of application of 6a,12a-dehydrorotenone (21 μg) and rotenonone (22 μg) resulted in peak weights of 233 mg and 17 mg. Neither compound separated from rotenone in the prescribed solvent system, although rotenonone was primarily in the lower part of a 6a,12a-dehydrorotenone-rotenonone spot. Depending on conditions for a particular plate, rotenonone may be just barely evident in samples containing 20% 6a,12a-dehydrorotenone.

λ_{max} 219 (4·45), 256 (4·17), 264 (4·11), 362 (4·51), 381 (4·42).

12-*Deoxy*-$\Delta^{12(12a)}$-*dehydrodeguelin* (**7**). Transparent plates of m.p. 166·0–168·5°; λ_{max} 258 (4·40), 291 (4·01), 303 (4·00), 364 (4·52), 383 (4·45).

Elliptone (**9**). Transparent needles of m.p. 156·0–158·0°; ν_{max} 1680, 1620, 1515, 1475, 1340; λ_{max} 238 (4·63), 275 (3·95).

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