REACTION OF ROTENOIDS WITH HYDRAZINE

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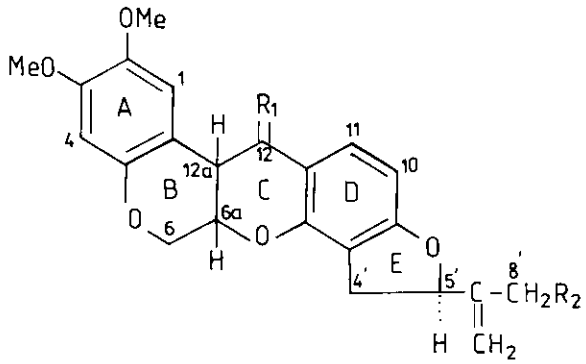
Abstract - The preparation and the structure of the rotenoid hydrazones is discussed. The normal hydrazones, prepared in acidic medium, retain the original rotenoid skeleton (6aβ,12aβ,5'β). The so-called isohydrazones, obtained in alkaline medium, are derivatives of the [1]benzopyrano[3,4-c] pyrazole ring system with cis B/C-rings juncture.

It is known[1] that treatment of rotenone 1 with reagents for carbonyl group (NH_2OH, $NH_2.NH_2$ or $C_6H_5NH.NH_2$) in methanolic KOH affords products called isoximes and isohydrazones, isomeric to the normal products obtained in acidic (AcOH) medium. The structure of [1]benzopyrano[4,3-d]isoxazole derivative has been assigned[2] in 1961 to the isoxime of 1 on the basis of its $FeCl_3$ reaction, UV and IR data, while the isohydrazones of 1 have been characterized[1] in 1928 only by their mp and $FeCl_3$ reaction.

Here we present the results on the preparation, structure determination and the biological activity of the isohydrazones of 1, amorphigenin 2, amorphin 3 and amorphigenin-β-D-glucopyranoside 4. For comparison the normal hydrazones of 1 and 2 have been also prepared. The work is connected with our interest in the chemistry and biological activity of natural rotenoids and their derivatives, as well as of some hydrazino-compounds[3,4,5].

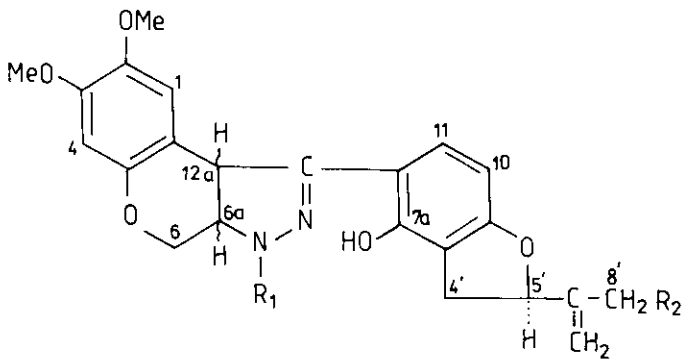
The normal hydrazones 5-6 were prepared in an acidic medium, while the isohydrazones were obtained in higher yields than the reported for 8 by treating an ethanolic solution of rotenoids 1-4 with hydrazine hydrate or methylhydrazine (see Experimental).

The IR spectra of all hydrazones 5-15 showed the absence of C_{12} carbonyl function and their mass spectra confirmed the expected molecular weights ($M^{+\cdot}$).



1. R_1 = O, R_2 = H
2. R_1 = O, R_2 = OH
3. R_1 = O, R_2 = O-Gl-O-Ar
4. R_1 = O, R_2 = O-Gl
5. R_1 = N.NH_2, R_2 = H
6. R_1 = N.NH_2, R_2 = OH
7. R_1 = N.NH.Ac, R_2 = H

The splitting pattern of 6-H_2, 6a and 12a protons, revealed by the ^{1}H NMR spectra (Table 1) of the normal hydrazones 5-7, was similar to that of the parent rotenoids 1-2, thus confirming their rotenoid skeleton with 6aß,12aß cis-fused B/C rings[6] (epimerization of rotenoids at 6a and 12a does not take place in an acidic medium[2]). The up-field shift of 1-H, C_2-OMe and 12a-H in the acetate 7 suggested the anti-orientation of NHAc group towards ring A and 12a-H.



8a,b: R_1 = H, R_2 = H
9a,b: R_1 = H, R_2 = OH
10a,b: R_1 = H, R_2 = O-Gl-O-Ar
11a,b: R_1 = H, R_2 = O-Gl
12a,b: R_1 = CH_3, R_2 = H
13a,b: R_1 = CH_3, R_2 = OH
14a,b: R_1 = CH_3, R_2 = O-Gl-O-Ar
15a,b: R_1 = CH_3, R_2 = O-Gl

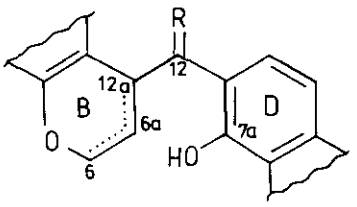
The 400 MHz spectrum (Table 1) of the isohydrazone 8 revealed the presence of two

D_2O-exchangeable protons at ~δ11.10 (7a-OH) and ~δ5.80 (NH). As expected, the N-methyl compounds 12 and 13 showed no signal for NH proton and all iso-compounds 8-15 gave $FeCl_3$ reaction for the phenolic C_{7a} hydroxyl. Full decoupling experiments of the protons at 6, 6a and 12a positions in 8 confirmed the proposed skeleton. The numbering of the rotenoid skeleton is retained. On irradiation of the 6a-H resonance at δ4.31(ddd, J=8.5, 2.3 and 1.0 Hz), the 12a-H doublet (δ4.63, J=8.5 Hz) collapsed to a singlet, while the two dd of 6-H_2 at δ4.45 (J=12.0, 2.3 Hz) and at δ4.21 (J=12.0, 1.0 Hz) appeared as doublets with J=12.0 Hz. The N-methyl compounds 12-13 exhibited similar ^1H NMR spectra (Table 1), although the 6a-H appeared at higher field, due to the shielding effect of NCH_3.

These data were consistent with the ring system of [1]benzopyrano[3,4-c]pyrazole 8-15 and ruled out any hydrazones 16 derived from the seco compounds 17 (the latter available in alkaline solutions[2] of rotenoids), as well as any cycloaddition product involving six- or four-membered ring C. The cis juncture of rings B and C was suggested by $J_{6a,12a}$=8.5 Hz and by the small coupling constants between 6a-H and 6-H_2 (J=2.3 and 1.0 Hz); if the B/C juncture is trans, one of the two $J_{6a,6}$ is expected to be large. The ^1H NMR spectra of compounds 8, 12-13 suggested no tautomeric pyrazole derivatives. However, the presence of two cis diastereomers a (6aβ, 12aβ, 5'β) and b (6aα,12aα,5'β) in a ratio ~1:1 was indicated by the doubling of some signals, i.e. of 1, 4', 5', 7', 8', NH, OH and OMe protons. Most probably, under the reaction conditions the first step is the conversion of the rotenoids to the seco ketones 17a, followed by the formation of the seco hydrazones 16a and their cyclization to the isohydrazones 8-15. This suggestion was supported by the failure of the normal hydrazone 5 to undergo isomerization to the iso-compound 8a,b by treatment with bases (see Experimental), an indication that the former is not intermediate. It is well known[2] that similar treatment of rotenoids causes racemization at 6a and 12a via the formation of the compounds 17.

Obviously, the reaction of rotenoids with hydrazine proceeds stereoselectively as an intramolecular cycloaddition of the intermediate seco hydrazones 16a.

The NMR spectra of the acetates (Py, Ac_2O) of the glycosides were not informative for the pyrasole ring system, because of overlapping of the glycosidic protons[7] and the nonaromatic protons of the aglycone part. However, they revealed the NAc (~δ2.38), 7a-OAc (~δ2.30) and the expected number of glycosidic OAc groups, thus indicating that the sugar moieties in the glycosides 10, 11, 14 and 15 remain intact.



16. R = N.NH_2; a. $\Delta^{6a,12a}$; b. $\Delta^{6,6a}$

17. R = O ; a. $\Delta^{6a,12a}$; b. $\Delta^{6,6a}$

Table 1. Chemical Shifts of Compounds 5-8, 12 and 13.

Protons	5	6	7	8	12	13
H-11	7.77d	7.77d	7.86d	7.48d	7.47d	7.44d
H-1	6.45s	6.45s	6.18s	6.74s 6.75s	6.75s 6.76s	6.74s
H-10	6.46d	6.46d	6.50d	6.52d	6.53d	6.52d
H-4	6.45s	6.45s	6.46s	6.45s	6.46s	6.42s
H-5'	5.14t	5.30t	5.18t	5.27t	5.29t	5.42t
H-7'	5.06s	5.30s	5.06s	5.10s 5.08s	5.11s	5.28s
H-7'	4.90s	5.24s	4.91s	4.93s 4.91s	4.90s	5.28s
H-12a	4.32d	4.31d	4.17d	4.63d	4.68d	4.76d
H-6a	4.50bs	4.49bs	4.54bs	4.31ddd	3.48ddd	3.46ddd
H-6'	4.65dd	4.62dd	4.64dd	4.45dd	4.46dd	4.44dd
H-6"	4.30dd	4.28dd	4.28dd	4.21dd	4.23dd	4.18- 4.36m
OMe	3.81s	3.81s	3.82s	3.80s	3.81s	3.80s
OMe	3.73s	3.74s	3.66s	3.57s 3.54s	3.59s 3.55s	3.56s
H_a-4'	3.27dd	3.32dd	3.27dd	3.36dd 3.33dd	3.36dd	3.42dd
H_b-4'	2.93dd	3.04dd	2.94dd	3.03dd 3.01dd	3.05dd	3.14dd
8'-CH_3 or 8'-CH_2	1.75s	4.15- 4.36m	1.76s	1.80s 1.75s	1.78s 1.76s	4.18- 4.36m
N-CH_3	-	-	-	-	2.98s	2.98s
NH	5.50	5.51	8.85s	5.80s 5.78s	-	-
7a-OH	-	-	-	11.12s 11.08s	11.13s 11.19s	11.20s 11.18s
NAc	-	-	2.45s	-	-	-

J in Hz - a. Common for all compds.: 10,11 ~8.5; $4'_a$,5' ~9.5; $4'_b$,5' ~8.5; $4'_a$,$4'_b$ ~16.0; b. Only for compds. 5-7: 6a,12a ~3.0; 6a,6' ~2.4; 6a,6" ~1.0; 6',6" ~12.0; c. Only for compds. 8, 12-13: 6a,12a ~8.5; 6a,6' ~2.3; 6a,6" ~1.0; 6',6" ~12.0.

This reaction presents a simple conversion of rotenoids and their glycosides to the corresponding [1]benzopyrano[3,4-c]pyrazole derivatives and offers easy access to the O-glycosyl-Δ^2-pyrazolines. The addition of diazoalkanes to coumarins with an electron-withdrawing substituent at position 3 produces Δ^1-pyrazolines[8] with the same [1]benzopyrano[3,4-c]pyrazole skeleton. Chalcones and flavanones react with hydrazine to give Δ^2-pyrazoline derivatives[9]. A survey of isohydrazones 8-11 for their anticancer and antimicrobial potency showed no activity (see Experimental).

EXPERIMENTAL

Melting points were measured on a Kofler hot stage microscope and are uncorrected; IR: Zeiss UR20 in KBr; UV: Zeiss Specord UV/VIS, ethanol; NMR: Bruker 400 for 8, and Bruker WM250 for the remaining compounds, in $CDCl_3$ and TMS as internal standard; MS: JEOL JMS D-300, 70eV.

n-Hydrazones 5-6: Prepared by treatment of an acidic (pH 5, 1ml gl AcOH) solution of the rotenoid (1mM) in EtOH (70ml) with 98% hydrazine hydrate (8mM). The reaction mixture was refluxed for 2-4 h and the separated solid filtered out from the hot solution. Attempts at a further purification of 5 and 6 by pTLC and crystallization *failed*. 5: *mp 226-227°C, 87% yield*; 6: *mp 227-228°C, 78% yield*; *acetate 7 (Ac_2O, room temperature)*: mp 204-205°C. UV, λ_{max}, nm - 5, 6: 243, 292 and 313; 7: 238, 245, 253, 297 and 322. IR, ν_{max}, cm^{-1} - 5, 6: 1625, 1600, 3387; 7: 1660, 1615.

Preparation of Isohydrazones 8-15 (Derivatives of [1]Benzopyrano[3,4-c]Pyrazole). General Procedure: To a boiling solution of the rotenoids 1-4 (1mM) in absolute EtOH (70ml), hydrazine hydrate (or methylhydrazine)(2mM) was added in portions. The reaction mixture was refluxed 2-4 h for hydrazine hydrate, or 4-6 h for methylhydrazine, and then cooled. The solid was filtered out and carefully washed with EtOH and ether.

All isohydrazones are ~1:1 mixtures of the cis- 6aα,12α and 6aβ,12aβ diastereomers (see NMR data, Table 1), not further separated because of their low stability and very close Rf, but with no additional TLC spots. The recorded mp's and spectra are valid for these diastereomeric mixtures. The isohydrazones 8-11 appeared unstable upon heating, exposure to light, crystallization and TLC monitoring. However, the NCH_3 isohydrazones 12-15 showed greater stability and their purification by pTLC and crystallization is possible. Melting points, yields - 8a,b: 231-235°C, 95%; 9a,b: 168-208°C, 80%; 10a,b: 228-232°C, 75%; 11a,b: 204-207°C, 95%; 12a,b: 195-209°C, 40%; 13a,b: 208-225°C, 52%; 14a,b: 138-162°C, 50%; 15a,b: 155-168°C, 15%. All hydra-

zones melt with decomposition.

UV, λ_{max} nm (log ε) - 8a,b: 243(0.32), 253(0.26), 295(0.65), and 322(0.40); 9a,b - 11a,b: 243, 253, 295 and 322; 12a,b - 15a,b: 243, 253, 300 and 318; IR, ν_{max} cm^{-1} - 8a,b - 9a,b: 1634, 1620, 1600, 3313, 3440 (broad band), and 3500 (only for 9a,b); 10a,b - 11a,b: 1634, 1615, 1600, 3313 and 3400 (broad band); 12a,b-13a,b: 1634, 1615, 1600, 3440 (broad band), and 3500 (broad band, only for 13a,b); 14a,b - 15a,b: 1634, 1615, 1600 and 3400 (broad band).

The MS spectra of all compounds (5-15a,b) in this study showed the expected $M^{+\cdot}$ (for the glycosides - M^{+} of aglycones) and fragments at m/z 192, 191 and 177.

Behaviour of n-Hydrazone 5 in Alkaline Medium: A reflux of the n-hydrazone 5 in alkaline EtOH (pH 9) for 4-5 h and TLC monitoring of the reaction mixture showed partial decomposition to a compound more polar than 5 and 8, but no conversion to the isohydrazone 8.

Antibacterial Tests: Due to the insolubility of the isohydrazones 8a,b - 10a,b in water, a 50mM/ml solution in DMSO was used in agar diffusion method on Petri dishes. The results were negative, with the following strains used: E. coli, S. lutea, S. aureus, strain 209P and its mutant UV-2.

Antitumour Tests: The antitumour activity of the rotenoids 1-3 and the isohydrazones 8a,b - 10a,b was tested on mice BDF 1 using the following strains: L 1210 leukemia, P388 leukemia, Lewiss lung carcinoma, and B-16 melanoma. The Tween 80 suspensions of the compounds were introduced intraperitoneally for five consecutive days, beginning 24 h after the transplantation. The following doses, based on preliminary toxicity determination, were applied: 1 - 4mg/kg, 8a,b - 300, 150 and 75mg/kg; 2 - 4mg/kg; 9a,b - 125, 63 and 31mg/kg; 3 - 32, 16 and 8mg/kg; 10a,b - 300, 150 and 75mg/kg. No activity was observed in all cases. The isohydrazones 8a,b - 10a,b were less toxic than the parent rotenoids 1-3.

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