907. *The Oxidation of Derivatives of* o-*Phenylenediamine. Part VI.*[1] *Reductive Acylation of Anilino*aposafranines *and Related Compounds.*

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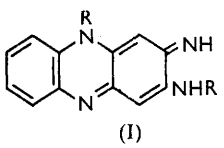
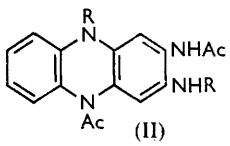
Anilino*apo*safranines and related compounds have a *p*-quinonoid system which is readily reduced. The products of catalytic hydrogenation have little colour but are rapidly reoxidised in air to their original state. Hydrogenation in the presence of acetic or propionic anhydride yields stable non-quinonoid compounds containing 1—3 acyl groups. The therapeutic effect of the " rimino "-compounds in mouse tuberculosis is completely lost on reductive diacylation, indicating that the *p*-quinonoid system is involved in the antimycobacterial activity. Two reduced monoacetylated rimino-derivatives retained activity *in vivo*: it is presumed that these compounds are deacetylated in the tissues, regenerating the original active compounds.

ANILINO*apo*SAFRANINE and the related phenazine derivatives described in previous papers of this series contain a *p*-quinonoid system which may be readily reduced, and the unstable reduced compounds are spontaneously re-oxidised in air. It has been suggested [2,3] that, among other factors, the antituberculosis activity of these compounds depends on the *p*-quinonoid system which enables them to become involved in the terminal stages of the respiratory metabolism of the bacillus. Further, the reduced compounds on re-oxidation liberate hydrogen peroxide which may also play a part in the antimicrobial activity. It was of interest therefore to stabilise the reduced non-quinonoid compounds with a view to ascertaining whether the altered molecules had lost the power to protect mice from the usual experimental tuberculosis infection.

Anilino*apo*safranine (I; R = Ph), on hydrogenation in acetic anhydride at room temperature and pressure in the presence of Adams catalyst, yields the diacetyldihydrophenazine (II; R = Ph). Its isomer (III; R = Ph, R′ = H) under similar conditions yields the diacetyl derivative (IV; R = Ph, R′ = H). The last compound, in boiling


(I)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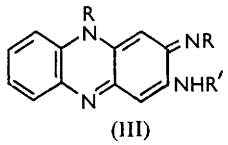
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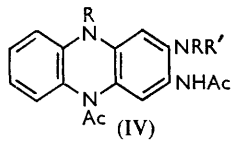
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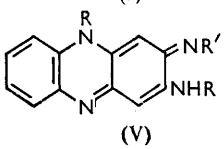
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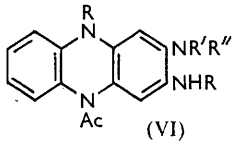
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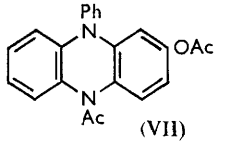
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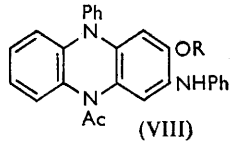
(VIII)

ethanolic sodium hydroxide, readily loses an acetyl group to give the monoacetyl derivative (III; R = Ph, R′ = Ac), identical with the compound resulting from the reaction of hot acetic anhydride with the amino-derivative (III; R = Ph, R′ = H). The " rimino "-compound [1] (V; R = R′ = Ph) forms only a monoacetyl derivative (VI; R = R′ = Ph, R″ = H), indicating that the $^{-}NH^{-}$ group of the arylamino-substituent is not acetylated under these conditions, and this has been confirmed with all the other compounds of this type investigated. On the other hand, when the hydrogen of the $^{=}NH$ group present in structure (I) is replaced by *iso*propyl, *cyclo*pentyl, or *cyclo*hexyl, reductive acetylation leads to the formation of a diacetyl derivative (VI; R = Ar, R′ = Ac, R″ = Pr^{i}, *cyclo*pentyl, or *cyclo*hexyl).

[1] Part V, Barry, Belton, O'Sullivan, and Twomey, *J.*, 1958, 859.
[2] Barry, Conalty, and Gaffney, *J. Pharm. Pharmacol.*, 1956, **8**, 1089.
[3] Barry, Belton, Conalty, Denneny, Edward, O'Sullivan, Twomey, and Winder, *Nature*, 1957, **179**, 1013.

The behaviour of compound (V; R = C_6H_4Cl-*p*, R′ = *cyclo*heptyl) however was exceptional: only one acetyl group entered the molecule, presumably on the reduced nitrogen at the 10-position to give compound (VI; R = C_6H_4Cl-*p*, R′ = *cyclo*heptyl, R″ = H). The failure to acetylate the N^3-position suggested that steric influences were responsible, and this was confirmed subsequently by the isolation of monoacetylated derivatives from compound (V; R = Ph) when R′ was *tert.*-butyl, and from compounds (V; R = C_6H_4Cl-*p*) when R′ was 2-methyl*cyclo*hexyl and *cyclo*octyl.

The monoacetyl derivative (VI; R = C_6H_4Cl-*p*, R′ = *cyclo*heptyl, R″ = H) may also be prepared by catalytic hydrogenation of the corresponding glyoxalinophenazine [1] in acetic anhydride, the glyoxalino-ring being opened at the same time. As expected the amino-compound (III; R = *cyclo*hexyl, R′ = H) yields a triacetyl derivative (IV; R = *cyclo*hexyl, R′ = Ac).

Hydrogenated *apo*safranone derivatives are also stabilised by acetylation. Thus, *apo*safranone gives the diacetylated compound (VII) whereas anilino*apo*safranone gives the monoacetyl derivative (VIII; R = H), acetylation of the phenolic hydroxyl being hindered probably by hydrogen-bonding with the 2-anilino-group. Further treatment of the hydroxy-compound (VIII; R = H) by warm acetic anhydride brings about acylation of the phenolic hydroxyl group, to give the diacetyl derivative (VIII; R = Ac). When propionic anhydride was used in the reductive acylation of the compounds (V; R = C_6H_4Cl-*p*, R′ = *cyclo*hexyl and *cyclo*heptyl) the results obtained were similar to those with acetic anhydride.

In most cases mild treatment with alkali of the reduced acetylated compounds regenerates the original quinonoid pigments. However, the diacetyl derivative (II; R = Ph) and the triacetyl derivative (IV; R = *cyclo*hexyl, R′ = Ac) are extensively decomposed by alkali; the acetoxy-compound (VII) regenerates *apo*safranone on treatment with sodium nitrite, and the phenol (VIII; R = H) is reconverted into anilino*apo*safranone on exposure to ultraviolet radiation. As already stated, compound (IV; R = Ph, R′ =H) may be hydrolysed by ethanolic alkali in two stages.

All the acylated reduced compounds are colourless, crystalline, and reasonably stable materials which gradually acquire a pink tinge. They appear to some extent to be light-sensitive. Of the compounds tested all had lost the ability on oral administration to protect mice from an intravenous infection with virulent tubercle bacilli, with the exception of the monoacetyl derivatives (VI; R = C_6H_4Cl-*p*, R′ = *cyclo*heptyl and 2-methyl-*cyclo*hexyl, R″ = H). It is probable that these two compounds are hydrolysed in the tissues to permit the regeneration of the *p*-quinonoid system.

Experimental

Light petroleum had b. p. 40—60°, ligroin had b. p. 100—120°.

5-*Acetyl*-2 : 3-*dianilino*-5 : 10-*dihydro*-10-cyclo*hexylphenazine*.—2-Anilino-3 : 5-dihydro-5-*cyclo*hexyl-3-phenyliminophenazine (0·9 g.), acetic anhydride (90 c.c.), and Adams catalyst (0·1 g.) were shaken in hydrogen for 3 hr. The mixture was poured into ice-water (1 l.), and the precipitate collected, washed with water, and dried in a vacuum-desiccator. The *monoacetyl derivative* crystallised from benzene–light petroleum as off-white needles (0·7 g.), m. p. 202—203° (Found: C, 79·7; H, 6·6; N, 10·6. $C_{32}H_{32}ON_4,\frac{1}{2}C_6H_6$ requires C, 79·7; H, 6·6; N, 10·6%).

2-*Acetoxy*-5-*acetyl*-5 : 10-*dihydro*-10-*phenylphenazine* (VII).—Reductive acetylation of *apo*safranone gave the *diacetyl compound* (VII) as off-white needles, m. p. 172—173° (Found: C, 74·1; H, 5·2; N, 7·7. $C_{22}H_{18}O_3N_2$ requires C, 73·7; H, 5·0; N, 7·8%).

5-*Acetyl*-3-*anilino*-5 : 10-*dihydro*-2-*hydroxy*-10-*phenylphenazine* (VIII; R = H).—Reductive acetylation of anilino*apo*safranone yielded the monoacetyl derivative in almost colourless fluffy needles, m. p. 210—212° (from chloroform–light petroleum) (Found: C, 76·0; H, 5·2; N, 10·3. $C_{26}H_{21}O_2N_3$ requires C, 76·7; H, 5·2; N, 10·3%).

2-*Acetoxy*-5-*acetyl*-3-*anilino*-5 : 10-*dihydro*-10-*phenylphenazine* (VIII; R = Ac).—The preceding compound, when heated with acetic anhydride on the water-bath for 20 min., gave the *diacetyl derivative*, m. p. 158—160° (from benzene–light petroleum) (Found: C, 75·1 H, 5·4; N, 9·5. $C_{28}H_{23}O_3N_3$ requires C, 74·8; H, 5·1; N, 9·4%).

2-p-*Chloroanilino*-5-p-*chlorophenyl*-5 : 10-*dihydro*-3-(N-cyclo*hexylpropionamido*)-10-*propionylphenazine*.—2-*p*-Chloroanilino-5-*p*-chlorophenyl-3 : 5-dihydro-3-*cyclo*hexyliminophenazine (1·5 g.), propionic anhydride (150 c.c.), and Adams catalyst (0·1 g.) were shaken in hydrogen for 3 hr. and poured into ice-water. The fine precipitate was washed with water and dried. The *dipropionyl compound* (0·8 g.), crystallised from benzene–light petroleum, had m. p. 235—236° (Found: C, 70·7; H, 5·8; N, 8·4; Cl, 10·6. $C_{36}H_{36}O_2N_4Cl_2,\frac{1}{2}C_6H_6$ requires C, 70·3; H, 5·85; N, 8·4; Cl, 10·65%).

2-p-*Chloroanilino*-5-p-*chlorophenyl*-5 : 10-*dihydro*-3-cyclo*heptylamino*-10-*propionylphenazine*.—2-*p*-Chloroanilino-5-*p*-chlorophenyl-3 : 5-dihydro-3-*cyclo*heptyliminophenazine was reductively propionylated and gave the *monopropionyl derivative* (almost white needles), m. p. 220—221° (Found: C, 70·9; H, 6·0; N, 8·9; Cl, 11·4. $C_{34}H_{34}ON_4Cl_2,\frac{1}{3}C_6H_6$ requires C, 70·7; H, 5·9; N, 9·2; Cl, 11·6%).

2″-*Methyl*-5 : 1′-*di*-(p-*chlorophenyl*)*glyoxalino*(5′ : 4′-2 : 3)*phenazine*-2′-spiro-1″-cyclo*hexane*.—2-Amino-4′-chlorodiphenylamine hydrochloride (5 g.), 2-methyl*cyclo*hexanone (3 c.c.), and ethanol (100 c.c.) were treated with *p*-benzoquinone (5 g.) in aqueous ethanol (2 : 1; 180 c.c.) at 30°. After 1 hr. the solution was made alkaline and diluted with water. The precipitate was dried and chromatographed in benzene on alumina. The *glyoxalinophenazine* was obtained as orange-yellow crystals (2·8 g.), m. p. 263—265°, from benzene (Found: C, 71·0; H, 5·0; N, 10·7; Cl, 13·6. $C_{31}H_{26}N_4Cl_2$ requires C, 70·9; H, 4·95; N, 10·7; Cl, 13·5%).

2-p-*Chloroanilino*-5-p-*chlorophenyl*-3 : 5-*dihydro*-3-(2-*methyl*cyclo*hexylimino*)*phenazine*.—Catalytic hydrogenation [1] of the above glyoxalino-compound yielded the "*rimino*"-*compound* as red needles, m. p. 203—204° (from ethanol) (Found: C, 70·5; H, 5·25; N, 10·7; Cl, 13·5. $C_{31}H_{28}N_4Cl_2$ requires C, 70·6; H, 5·3; N, 10·6; Cl, 13·5%).

5 : 1′-*Di*-(p-*chlorophenyl*)*glyoxalino*(5′ : 4′-2 : 3)*phenazine*-2′-spirocyclo-1″-*octane*.—2-Amino-4′-chlorodiphenylamine hydrochloride (7 g.), *cyclo*octanone (3 g.), and ethanol (120 c.c.) were treated with *p*-benzoquinone (7 g.) in aqueous ethanol (2 : 1; 200 c.c.) at 30°. The *compound* was purified as before, yielding orange crystals (0·8 g.), m. p. 265—266°, from benzene–ligroin (Found: C, 71·4; H, 5·3; N, 10·3; Cl, 13·4. $C_{32}H_{28}N_4Cl_2$ requires C, 71·2; H, 5·2; N, 10·4; Cl, 13·2%).

2-p-*Chloroanilino*-5-p-*chlorophenyl*-3 : 5-*dihydro*-3-cyclo*octyliminophenazine*.—Catalytic hydrogenation of the above glyoxalinophenazine yielded dark-red needles, m. p. 221—222° (Found: C, 71·1; H, 5·35; N, 10·45; Cl, 13·0. $C_{32}H_{30}N_4Cl_2$ requires C, 71·0; H, 5·5; N, 10·35; Cl, 13·1%).

Acetylated derivatives of 5 : 10-*dihydrophenazine*.

		Found (%)					Required (%)			
	M. p.	C	H	N	Cl	Formula	C	H	N	Cl
(II) R = Ph	255° *	74·8	5·4	11·9	—	$C_{28}H_{24}O_2N_4$	75·0	5·4	12·5	—
Compounds (IV)										
R = *cyclo*Hexyl; R′ = Ac	273—274	71·6	7·4	11·1	—	$C_{30}H_{38}O_3N_4$	71·7	7·6	11·1	—
R = Ph; R′ = H	120 *	75·8	5·6	11·7	—	$C_{28}H_{24}O_2N_4,\frac{1}{3}C_6H_6$	75·9	5·5	11·8	—
Compounds (VI; R = Ph)										
R′ R″										
Bu^t H	226—227	78·5	6·2	11·3	—	$C_{30}H_{30}ON_4,\frac{1}{3}C_6H_6$	78·7	6·6	11·5	—
*cyclo*Hexyl Ac	209—210	77·6	6·5	9·7	—	$C_{34}H_{34}O_2N_4,\frac{1}{3}C_6H_6$	77·7	6·5	10·1	—
Ph H	300	79·0	5·5	11·1	—	$C_{32}H_{26}ON_4$	79·7	5·4	11·6	—
Compounds (VI; R = C_6H_4Cl-*p*)										
R′ R″										
Pr^i Ac	201—202	69·3	5·6	9·1	11·1	$C_{31}H_{28}O_2N_4Cl_2$	69·7	5·3	8·6	11·1
*cyclo*Pentyl Ac	115—116	69·0	5·3	9·0	11·3	$C_{33}H_{30}O_2N_4Cl_2,\frac{1}{2}C_6H_6$	69·2	5·3	9·0	11·3
2-Me-*cyclo*hexyl H	190 *	69·6	5·9	9·6	12·4	$C_{33}H_{32}ON_4Cl_2$	69·4	5·6	9·8	12·4
*cyclo*Heptyl H	187—189	70·7	5·7	9·3	11·7	$C_{33}H_{30}ON_4Cl_2$	70·6	5·4	9·4	11·9
*cyclo*Octyl H	171—172	70·9	6·0	8·7	11·1	$C_{34}H_{34}ON_4Cl_2,\frac{1}{2}C_6H_6$	71·2	5·9	9·0	11·4

* Decomp.

The data for the remaining *compounds* are tabulated. Acetyl determinations were unsatisfactory and not reproducible.

The biological results will be published elsewhere.

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