385. *The Nature of the Porter–Silber Reaction.*

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The Porter–Silber reaction has been shown to involve acid-catalysed rearrangement of the adrenocortical side chain to a 20,21-glyoxal grouping followed by formation of the 21-(mono)phenylhydrazone. A compound containing the typical Porter–Silber chromophore has been isolated in crystalline form and shown to undergo a reversible mutarotation at room temperature. The reason for this mutarotation has been investigated and its solvent-dependence briefly studied.

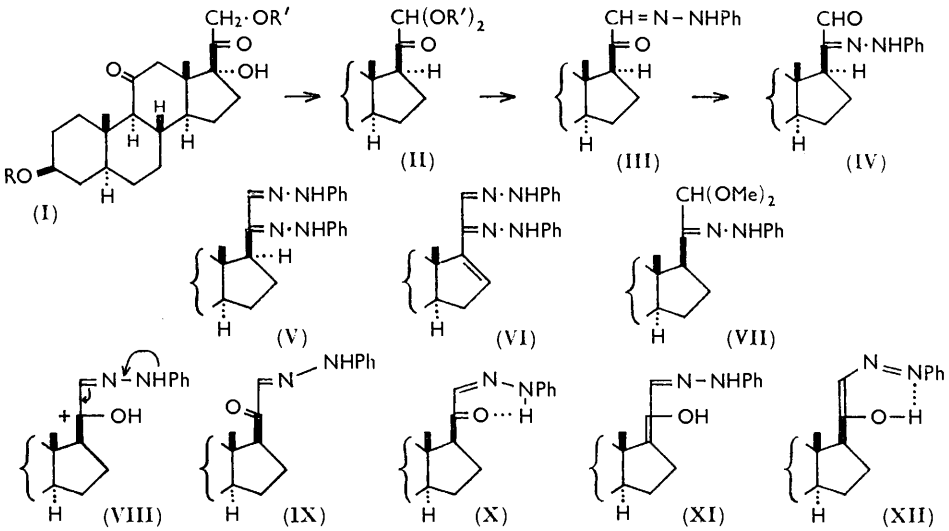
Treatment of steroids containing the adrenocortical side chain (as in I) with phenylhydrazine in diluted sulphuric acid produces a characteristic absorption at about 410 mμ.[1] This procedure has become an important method for the detection and determination of adrenocortical steroids in biological materials and is generally known as the Porter–Silber reaction.[1] To our knowledge there has been no definitive work on the constitution of the Porter–Silber chromophore although Walsen and Schlunk [2] recently proposed that it is as in (VI). Since the significance of the reaction should not be judged without a knowledge of the structures we examined the matter further. The present paper shows that the Porter–Silber chromophore is represented by the partial structure (III).

It seemed to us *a priori* that the chromophore would be derived from the glyoxal grouping (as II; R′ = H) itself formed by the type of rearrangement of the adrenocortical side chain under acid conditions observed by Mattox.[3] To test this hypothesis 21-acetoxy-3β,17α-dihydroxy-5α-pregnane-11,20-dione (I; R = H, R′ = Ac), an intermediate in the synthesis of the adrenocortical hormones,[4] was treated with methanolic hydrogen chloride [3] to give 3β-hydroxy-21,21-dimethoxy-5α-pregnane-11,20-dione (II; R = OH, R′ = Me). When kept at room temperature under the conditions of the Porter–Silber reaction [1] this acetal afforded 3β-hydroxy-11,20,21-trioxo-5α-pregnane 21-phenylhydrazone (III; R = H), characterised as its 3-acetate. The phenylhydrazone (III; R = H), on dissolution in diluted sulphuric acid, showed the characteristic maximum of the Porter–Silber chromophore. More importantly, when the Porter–Silber reaction was applied directly to the acetate (I; R = H, R′ = Ac) and the product was acetylated and filtered through alumina the same acetate phenylhydrazone (III; R = Ac) was obtained. The Porter–Silber colour developed at least thirty times faster when the test was applied to the acetal (II; R = H, R′ = Me) than when it was applied to the intact adrenocortical side chain as in (I; R = H, R′ = Ac).

In principle the monophenylhydrazone obtained in these reactions could be a derivative of the 21-aldehyde or of the 20-ketone (as IV). For comparison, pyruvaldehyde phenylhydrazone [5] was shown to have a band at 393 mμ in diluted sulphuric acid, comparable with that at 408 mμ shown by the 21-hydrazone (III; R = H). More significant was the following experiment. When the dimethyl acetal (II; R = H, R′ = Me) was treated

[1] Porter and Silber, *J. Biol. Chem.*, 1950, **185**, 201; 1954, **210**, 923.
[2] Walsen and Schlunk, *Experientia*, 1959, **15**, 71.
[3] Mattox, *J. Amer. Chem. Soc.*, 1952, **74**, 4340.
[4] Evans, Hamlet, Hunt, Jones, Long, Oughton, Stephenson, Walker, and Wilson, *J.*, 1956, 4356.
[5] Reynolds and van Allan, *Org. Synth.*, 1952, **32**, 84.

with phenylhydrazine hydrochloride and sodium acetate in aqueous methanol at room temperature, it afforded a yellow precipitate having 280 mμ (ε 11,400) indicative of the saturated phenylhydrazone chromophore (as VII). On acetylation with pyridine–acetic anhydride and working up in the usual way there resulted the monophenylhydrazone (IV;



R = Ac) having $\lambda_{max.}$ 340 mμ (ε 15,800). The Porter–Silber chromophore (III; R = Ac) exhibited a comparable maximum in ethanol at 350 mμ but of much greater intensity (ε 27,500). In addition the two compounds (III; R = Ac) and (IV; R = Ac) were different in every other physical property.

When warmed with phenylhydrazine hydrochloride and sodium acetate in aqueous ethanol the dimethyl acetal (II; R = H, R′ = Me) gave the osazone (V; R = H). This was also obtained from the 21-hydrazone (III; R = H) in the same conditions.

It appears that in the strongly acidic conditions of the Porter–Silber reaction protonation of the chromophore (as III) to give a chromophore (as VIII), responsible for the characteristic maximum, prevents further reaction with phenylhydrazine. In consequence an osazone is not, in fact, formed under the normal conditions of the Porter–Silber reaction.

The acetate phenylhydrazone (III; R = Ac) showed a large negative rotation ($[\alpha]_D$ −150°) in CCl_4 which changed in 6 hr. to a positive value (+125°). Removal of the solvent and crystallisation from methanol gave back the original material with the large negative rotation. The solvent-dependence of this phenomenon was then examined with the results summarised in the Table. All solutions were kept in the dark at room temperature (23° ± 3°).

Solvent	Concn. (*c*)	Initial $[\alpha]_D$	$[\alpha]_D$ after various times (in parentheses)
CCl_4	0·56	−150°	+125° (6 hr.), +141° (3 days)
$CHCl_3$	0·79	−128	+38 (3 hr.), +39 (2 days)
,,	0·53	−128	+38 (2 hr.)
,,	1·10	−127	+30 (6 hr.)
C_6H_6	0·75	−192	−29° (11 days)
Tetrahydrofuran	0·55	−126	−120 (18 hr.), −113 (24 days)
EtOAc	0·89	−172	−134 (24 hr.), −113 (15 days)
Pyridine	0·85	−206	−206 (24 hr.)
MeOH	0·96	−227	−201 (24 hr.)

The simplest interpretation of these results is that a solvent-dependent equilibrium is set up between the *trans*- (IX) and the *cis*-configuration (X) for the monophenylhydrazone. In well-solvating solvents the *trans*-isomer is stabilised whereas in poorly

solvating solvents " internal solvation " is favoured through formation of a strong (O–N–H) hydrogen bond. The evidence for this is briefly as follows. Although the mutarotated carbon tetrachloride solution showed little change in its ultraviolet absorption spectrum it gave a significantly different infrared spectrum. The initial solution showed the usual bands for the 3β-acetate and 11-ketone as well as a sharply defined N–H band at 3240 cm.$^{-1}$. The 20-carbonyl group gave (in CCl_4) a band at 1660 cm.$^{-1}$ as would be expected for an αβ-unsaturated β-hydrazino-ketone chromophore.[6] After mutarotation the NH band had vanished and the 20-ketone band had largely disappeared. This result appears to exclude enolisation to a system such as (XI), since the NH band would then be retained. Besides the hydrogen-bonded ketone formula (X), one must also consider the enolic form (XII). These are distinct molecular species whose relative proportions are known to be solvent-dependent.[7] However, it seems most improbable that an intramolecular proton-transfer from nitrogen to oxygen, (X) ⟶ (XII), could be so strongly activated at room temperature as to require several hours for completion.[8] We do not consider, therefore, that the rearrangement of (X) to (XII) can represent the process under investigation. The mutarotation appears to be best explained by a rearrangement of the *trans*- (IX) to the *cis*-hydrazone (X), which can exist as (X) or as (XII) or as a mixture of the two (see above).

Experimental

M. p.s were taken on the Kofler block. Ultraviolet absorption spectra were, unless specified to the contrary, determined for ethereal solutions with the Unicam S.P. 500 spectrophotometer. Infrared spectra were determined for Nujol mulls. Light petroleum refers to the fraction of b. p. 60—80°. $[\alpha]_D$ are for $CHCl_3$ solutions unless indicated otherwise. The expression " diluted sulphuric acid " refers to a solution made 31 : 19 (v/v) sulphuric acid–water.

3β-*Hydroxy*-21,21-*dimethoxy*-5α-*pregnane*-11,20-*dione*.—This *compound* (II; R = H; R′ = Me) was prepared from 21-acetoxy-3β,17α-dihydroxy-5α-pregnane-11,20-dione by the general procedure of Mattox.[3] Recrystallised from methanol it had m. p. 150—151°, $[\alpha]_D$ +109° (*c* 1·47) (Found: C, 70·35; H, 9·2; OMe, 17·8. $C_{23}H_{36}O_5$ requires C, 70·4; H, 9·2; OMe, 15·8%).

3β-*Hydroxy*-11,20,21-*trioxo*-5α-*pregnane* 21-*Phenylhydrazone*.—The preceding acetal (50 mg.) in ethanol (2·5 ml.) was added to a 1% solution of phenylhydrazine hydrochloride in diluted sulphuric acid (5 ml.). The resulting solution, which rapidly became orange-coloured, was left at room temperature for 30 min. Dilution with water gave a yellow precipitate (40 mg.) which, on crystallisation from methanol, had m. p. 182—185°. Analysis showed solvation by methanol. Further crystallisation from acetic acid then gave the 21-*phenylhydrazone*, m. p. 213—215°, $[\alpha]_D$ −158° (*c* 1·03), +8° (after 24 hr), $\lambda_{max.}$ 238, 290, 296, and 350 mμ (ε 13,900, 4700, 5300, and 23,700 respectively), $\lambda_{max.}$ [in diluted sulphuric acid–ethanol (2 : 1)] 253, 408 mμ (ε 6600 and 23,800 respectively), $\nu_{max.}$ 1695 (11-C=O), 1631 (conjugated 20-C=O) and 1600 (aromatic C=C) cm.$^{-1}$ (Found: C, 74·0; H, 8·0; N, 7·05. $C_{27}H_{36}N_2O_3$ requires C, 74·3; H, 8·3; N, 6·4%).

The phenylhydrazone was treated with pyridine–acetic anhydride overnight at room temperature, to give the 3β-*acetate*. Recrystallised from methanol this had m. p. 144—146°; recrystallised from acetic acid it had m. p. 139—141°, $[\alpha]_D$ −128° (*c* 0·53), +38° (after 2 hr.), $\lambda_{max.}$ 238, 290, 298, and 350 mμ (ε 14,400, 5500, 5800, and 27,500 respectively), $\nu_{max.}$ 1720 (OAc and acetic acid carboxyl), 1701 (11-C=O), 1633 (conjugated 20-C=O) and 1603 (aromatic C=C) cm.$^{-1}$ (Found: C, 68·75; H, 7·75. $C_{29}H_{38}N_2O_4,CH_3{\cdot}CO_2H$ requires C, 69·1; H, 7·85%). Titration with 0·5N-sodium hydroxide in ethanol confirmed the presence of 1 mol. of acetic acid.

[6] See, *inter al.*, Cromwell, Miller, Johnson, Frank, and Wallace, *J. Amer. Chem. Soc.*, 1949, **71**, 3337; Hadži, *J.*, 1956, 2143; Weinstein and Wyman, *J. Org. Chem.*, 1958, **23**, 1618; Stachel, *Chem. Ber.*, 1960, **93**, 1059.

[7] Burawoy, Salem, and Thompson, *J.*, 1952, 4793; Burawoy, Cais, Chamberlain, Liversedge, and Thompson, *J.*, 1955, 3721, 3727.

[8] See, for example, Swain and Labes, *J. Amer. Chem. Soc.*, 1957, **79**, 1084, and references there cited.

The 3β-acetate phenylhydrazone (III; R = Ac) (30 mg.) in ethanol (0·8 ml.) and 15% aqueous potassium carbonate (0·2 ml.) was shaken (suspension) for 48 hr. The ethanol was removed *in vacuo* and the residue extracted with chloroform. Crystallisation from methanol and then from glacial acetic acid gave the 3β-hydroxy-phenylhydrazone (III; R = H), identified by m. p., mixed m. p., and ultraviolet and infrared spectra. It showed $[\alpha]_D$ −157° (*c* 0·78), +8° (after 24 hr.).

The 3β-acetate phenylhydrazone (III; R = Ac) (10 mg.) was allowed to mutarotate at room temperature in CCl_4 from $[\alpha]_D$ −150° to $[\alpha]_D$ +125° (6 hr.). The solvent was removed *in vacuo* and the residue crystallised once from methanol to give back the original acetate, identified by m. p., mixed m. p., and rotation. In a second experiment the 3β-acetate phenyl hydrazone (91 mg.), which had mutarotated to $[\alpha]_D$ +129°, was worked up to give starting material in three crops (54 + 23 + 12 mg.). The identity of each crop was checked by m. p., mixed m. p., rotation, and mutarotation.

A portion (69 mg.) of the product (368 mg.) from a Porter–Silber reaction with 21-acetoxy-3β,17α-dihydroxy-5α-pregnane-11,20-dione (399 mg.) was treated at room temperature with pyridine–acetic anhydride for 18 hr. The derived acetate was filtered in benzene–light petroleum (1 : 1) through alumina (Grade V) and then crystallised from methanol, to furnish the acetate phenylhydrazone (III; R = Ac) characterised as above. The identity was established by m. p., mixed m. p., and ultraviolet and infrared spectra.

Pyruvaldehyde Phenylhydrazone.—This compound [5] had $\lambda_{max.}$ 237, 295, and 344 mμ (ε 12,700, 4600, and 21,600 respectively), $\lambda_{max.}$ [in diluted sulphuric acid–ethanol (2 : 1)] 393 mμ (ε 25,400), $\nu_{max.}$ 1644 (conjugated C=O) and 1600 (aromatic C=C) cm.$^{-1}$.

3β-*Hydroxy*-11,20,21-*trioxo*-5α-*pregnane Bisphenylhydrazone.*—3β-Hydroxy-21,21-dimethoxy-5α-pregnane-11,20-dione (100 mg.) with phenylhydrazine hydrochloride (500 mg.) and hydrated sodium acetate (500 mg.) in water (4 ml.) was warmed with the minimum of ethanol to give a solution and then refluxed for 15 min. The yellow crystalline precipitate which separated (110 mg.) had m. p. 243—250°. Recrystallisation from chloroform–methanol afforded the desired *phenylosazone* (V; R = H), m. p. 250—254°, $[\alpha]_D$ −72° (*c* 1·00), $\lambda_{max.}$ 257, 310, and 395 mμ (ε 18,600, 11,000, and 17,700 respectively), $\lambda_{max.}$ [in diluted sulphuric acid–ethanol (2 : 1)] 260 and 416 mμ (ε 10,900 and 25,400 respectively), $\nu_{max.}$ 1695 (11-C=O) and 1592 (aromatic C=C) cm.$^{-1}$ (Found: C, 74·7; H, 7·95; N, 10·15. $C_{33}H_{42}N_4O_2$ requires C, 75·25; H, 8·05; N, 10·65%).

The osazone was also obtained by refluxing 3β-hydroxy-11,20,21-trioxo-5α-pregnane 21-phenylhydrazone (84 mg.) with phenylhydrazine hydrochloride (200 mg.) and hydrated sodium acetate (200 mg.) in the minimum of aqueous ethanol. After 4 hours' refluxing the crystalline osazone was filtered off (70 mg.) and identified by m. p. and mixed m. p.

3β-*Acetoxy*-11,20,21-*trioxo*-5α-*pregnane* 20-*Phenylhydrazone* (IV; R = Ac).—3β-Hydroxy-21,21-dimethoxy-5α-pregnane-11,20-dione (180 mg.) in methanol (10 ml.) was added to a solution of phenylhydrazine hydrochloride (90 mg.) and hydrated sodium acetate (90 mg.) in water (5 ml.), and the solution left for 2 hr. at room temperature. Dilution with water gave a yellow precipitate (140 mg.), $\lambda_{max.}$ 280 mμ (ε 11,400), indicative of the saturated phenylhydrazone (VII; R = H). The precipitate was treated with pyridine (1·0 ml.) and acetic anhydride (0·5 ml.) overnight at room temperature. The product was chromatographed over alumina (Grade V; 30 g.). Elution with benzene–light petroleum (1 : 1) gave the desired 21-*aldehyde* 20-*phenylhydrazone* (IV; R = Ac) (60 mg.). Recrystallised from chloroform–methanol this had m. p. 221—223°, $[\alpha]_D$ +24° (*c* 1·02), $\lambda_{max.}$ 236 and 340 mμ (ε 11,000 and 15,800 respectively), $\lambda_{infl.}$ 298 mμ (ε 5100), $\nu_{max.}$ 1720 (OAc), 1698 (11-C=O), 1645 (conjugated 21-CHO) and 1597 (aromatic C=C) cm.$^{-1}$ (Found: C, 72·65; H, 8·1; N, 6·1. $C_{29}H_{38}N_2O_4$ requires C, 72·75; H, 8·0; N, 5·85%). The infrared spectrum of this compound in the " finger-print " region was very different from that of the isomer (III; R = Ac).

Comparison of Rate of Development of Porter–Silber Colour.—The rates of chromogen formation from 21-acetoxy-3β,17α-dihydroxy- and from 3β-hydroxy-21,21-dimethoxy-5α-pregnane-11,20-dione were compared as follows. The compound (10 mg.) in ethanol (2·5 ml.) was mixed with 1% phenylhydrazine hydrochloride solution in diluted sulphuric acid (5 ml.) until homogeneous at room temperature. At definite intervals aliquot parts (0·1 ml.) were removed, the appropriate amount of diluted sulphuric acid was added, and the absorption intensity at 408 mμ determined. The appropriate control runs were made at the same time. The Table shows the resulting optical densities at 408 mμ in arbitrary units.

Time (hr.)	0·33	1	1·5	2·5	3·5
17α-OH, 21β-OAc	0·01	0·04	0·06	0·10	0·13
17α-H, 21,21-$(OMe)_2$	0·33	0·38	0·37	0·38	0·37

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