

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF STANFORD UNIVERSITY, STANFORD, CALIF.]

Optical Rotatory Dispersion Studies. LXXXV.<sup>1</sup> Circular Dichroism and Optical Rotatory Dispersion of the Nitrite and Nitro Chromophores<sup>2</sup>

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Parallel optical rotatory dispersion, circular dichroism, and ultraviolet absorption measurements have been conducted on a series of steroids containing the nitrite and nitro chromophores. The nitrite grouping exhibits multiple O.R.D. Cotton effects and C.D. curves in the 350–400  $m\mu$  region, which lend themselves readily to stereochemical assignments of the corresponding hydroxyl function—other optically active chromophores such as saturated carbonyl functions offering no complications. Particularly instructive results were obtained with the nitro chromophore, where O.R.D. and C.D. measurements clarify greatly the location of the relevant electronic transitions, which are often hidden in the presence of other chromophores, such as the carbonyl function, and which frequently cannot be detected by ultraviolet spectral examination. In the present studies either O.R.D. or C.D. offered essentially the same information, although occasionally one or the other method appeared to be preferable in the detection of overlapping absorption bands.

In three earlier articles,<sup>3–5</sup> we have compared the relative advantages of optical rotatory dispersion and circular dichroism measurements among organic compounds possessing the C=S<sup>3</sup> or C=O<sup>3,4,6</sup> chromophores or the inherently dissymmetric chromophore of the biphenyls and binaphthyls.<sup>5</sup> It was concluded that, while for the majority of structural and stereochemical applications in organic chemistry both methods will afford the necessary information, there are certain problems where either rotatory dispersion or circular dichroism alone is to be preferred. These special situations will be uncovered only if comparative rotatory dispersion and circular dichroism studies are performed—invariably in conjunction with the appropriate spectral absorption measurements—and in the present paper we record such work for the nitrite and nitro chromophores.

## The Nitrite Chromophore

The only relevant studies, prior to our own work, are the classical investigations of Kuhn,<sup>8</sup> who examined the optical rotatory dispersion, circular dichroism, and ultraviolet absorption of three secondary nitrites of the type RCH(CH<sub>3</sub>)ONO (R = C<sub>6</sub>H<sub>5</sub>, C<sub>6</sub>H<sub>11</sub>, and C<sub>6</sub>H<sub>13</sub>). In the region of absorption (350–400  $m\mu$ ) of the nitrite chromophore, they observed a series of well-defined Cotton effects, which were in good agreement with the corresponding circular dichroism curves. No further work was done in this field and this interesting anomalous dispersion behavior has not been employed for stereochemical purposes since that time,<sup>9</sup> presumably because of instability of such alkyl nitrites. The recent extensive studies by Barton and collaborators<sup>10</sup> on the photochemistry of steroidal nitrites demonstrated that such crystalline nitrites are easily prepared and manipulated. Consequently, there was undertaken in our laboratory<sup>11</sup> an extensive optical rotatory dispersion

survey of such steroidal nitrites, from which it was concluded<sup>11</sup> "that nitrites should be considered seriously as suitable 'chromophoric' derivatives of alcohols and that their multiple Cotton effects can be used for purposes of differentiation between epimeric pairs of alcohols."

We have now selected certain test cases from the earlier work<sup>11</sup>—either because of their particular relevance to stereochemical studies or because of the presence of two different optically active chromophores—and subjected them to comparative circular dichroism measurements.<sup>12</sup>

In Fig. 1 and 2 are reproduced the O.R.D., C.D., and ultraviolet absorption curves of cholestan-3 $\beta$ -ol nitrite (I) and its epimer, cholestan-3 $\alpha$ -ol nitrite (II). As noted earlier,<sup>11</sup> the rotatory dispersion curve of the  $\beta$ -isomer I is characterized by a series of positive Cotton effects, while that (Fig. 2) of the  $\alpha$ -isomer II shows consecutive negative Cotton effects, superimposed on a positive background. The amplitude of these Cotton effects is weak, and the effect of the background rotation of the parent system becomes very noticeable. Thus, the spirostan system of the steroidal sapogenins is known<sup>13</sup> to exhibit a strong negative background rotation and it is not surprising, therefore, that the multiple negative Cotton effects of a  $3\alpha$ -nitrite of the  $5\alpha$ -spirostan series are now found on the negative side, in contrast to the situation observed (Fig. 2) with the analogous nitrite II of the cholestan series. Once these background effects are appreciated, then no difficulty will be encountered in using Cotton effects of even such low rotational strength for stereochemical purposes. The circular dichroism curves of I and II correspond very well to the O.R.D. and ultraviolet absorption curves and show the expected fine structure. The amplitude of the multiple and partially overlapping circular dichroism bands is so low that in Fig. 2 the individual absorption bands are more clearly recognized in the O.R.D. than the C.D. curve, a state of affairs which is rather unusual. In Fig. 1, the fine structure is equally well resolved in both the C.D. and O.R.D. curves and certainly illustrates the advantage of these two optical methods for the clear definition of overlapping absorption bands, which in several instances are only reflected in shoulders or inflections of the ultraviolet absorption spectra (Fig. 1 and 2).

In contrast to the weak amplitudes of O.R.D. and C.D. curves of steroidal 3-nitrites (I, II), 20-nitrites<sup>11</sup> show a tenfold increase. The O.R.D., C.D., and ultra-

(1) Paper LXXXIV: K. Wellman, E. Bunnenberg, and C. Djerassi, *J. Am. Chem. Soc.*, **85**, 1870 (1963).

(2) Supported by the National Science Foundation (grant No. G 19905) and the National Cancer Institute (grant No. CRTV-5061) of the National Institutes of Health, U. S. Public Health Service.

(3) C. Djerassi, H. Wolf, and E. Bunnenberg, *J. Am. Chem. Soc.*, **84**, 4552 (1962).

(4) C. Djerassi, H. Wolf, and E. Bunnenberg, *ibid.*, **85**, 324 (1963).

(5) K. Mislow, E. Bunnenberg, R. Records, K. Wellman, and C. Djerassi, *ibid.*, **85**, 1342 (1963).

(6) L. Velluz and M. Legrand, *Angew. Chem.*, **73**, 603 (1961), have measured the circular dichroism curves of a substantial number of steroid ketones, the rotatory dispersion curves of which had already been reported earlier.<sup>7</sup>

(7) C. Djerassi, "Optical Rotatory Dispersion; Applications to Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1960.

(8) W. Kuhn and H. Biller, *Z. physik. Chem. (Leipzig)*, **B29**, 1 (1935).

(9) See also K. Freudenberg and H. Biller, *Ann.*, **510**, 230 (1934).

(10) For recent reviews of this work, see A. L. Nussbaum and C. H. Robinson, *Tetrahedron*, **17**, 35 (1962); J. M. Erikson and D. L. Forbess in C. Djerassi, Ed., "Steroid Reactions," Holden-Day, San Francisco, Calif., 1963 Chapter 8.

(11) C. Djerassi, I. T. Harrison, O. Zagneetko, and A. L. Nussbaum, *J. Org. Chem.*, **27**, 1173 (1962).

(12) After completion of the present work, there appeared a brief note by M. Legrand and R. Viennet, *Compt. rend.*, **225**, 2985 (1962), in which the circular dichroism behavior of some steroidal nitrites is mentioned.

(13) C. Djerassi and R. Ehrlich, *J. Am. Chem. Soc.*, **78**, 440 (1956).

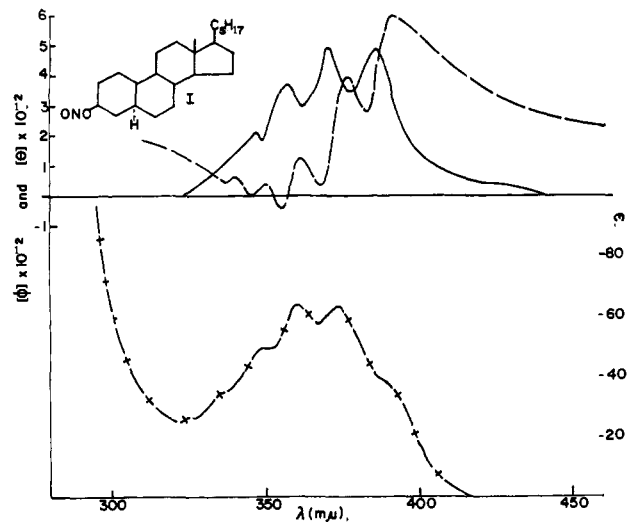


Fig. 1.—Circular dichroism (—), optical rotatory dispersion (---), and ultraviolet absorption (-+ -+) curves of cholestan-3β-ol nitrite (I) in dioxane-pyridine (500:1) solution.

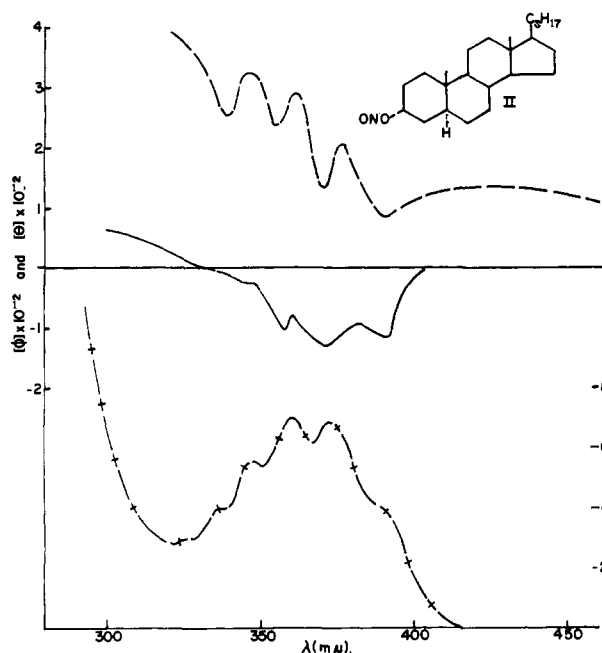


Fig. 2.—Circular dichroism (—), optical rotatory dispersion (---), and ultraviolet absorption (-+ -+) curves of cholestan-3α-ol nitrite (II) in dioxane-pyridine (500:1) solution.

violet curves of one representative, 5α-pregnan-20α-ol nitrite (III), are reproduced in Fig. 3 and there is excellent correspondence between these three curves. For stereochemical purposes, the most noteworthy feature is the series of four positive Cotton effects and positive C.D. maxima in the 350–400 mμ region, since exactly the opposite picture is exhibited (Fig. 4) by the C-20 epimer 5α-pregnan-3β,20β-diol 3-acetate 20-nitrite (IV).

The stereochemical utility of this information is demonstrated in Fig. 5 by the O.R.D. and C.D. curves of Δ<sup>4</sup>-pregnen-20β-ol-3-one nitrite (V). In addition to the 20β-nitrite function, this substance also contains the optically active Δ<sup>4</sup>-3-keto grouping,<sup>14</sup> as exemplified by the free alcohol Δ<sup>4</sup>-pregnen-20β-ol-3-one (VI). This substance (VI) exhibits (Fig. 6) a series of negative Cotton effects and negative C.D. maxima in the 310–

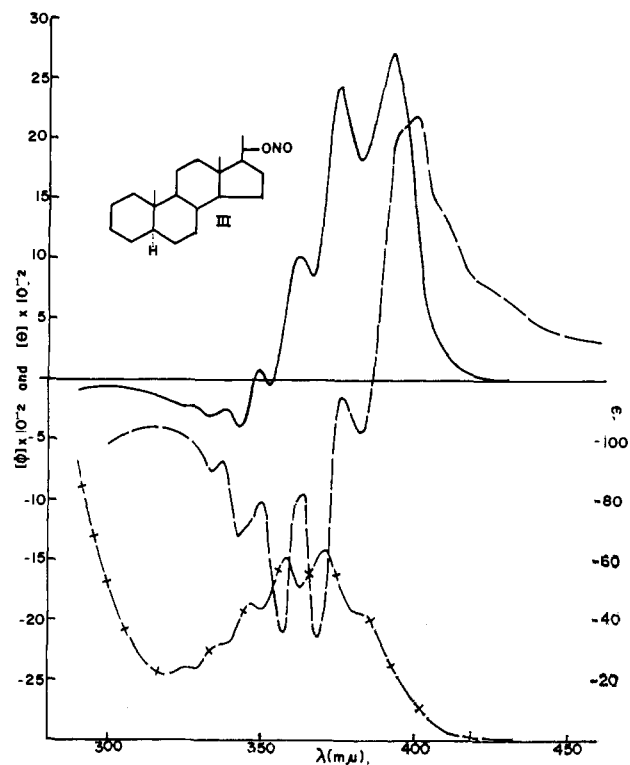


Fig. 3.—Circular dichroism (—), optical rotatory dispersion (---), and ultraviolet absorption (-+ -+) curves of 5α-pregnan-20α-ol nitrite (III) in dioxane-pyridine (500:1) solution.

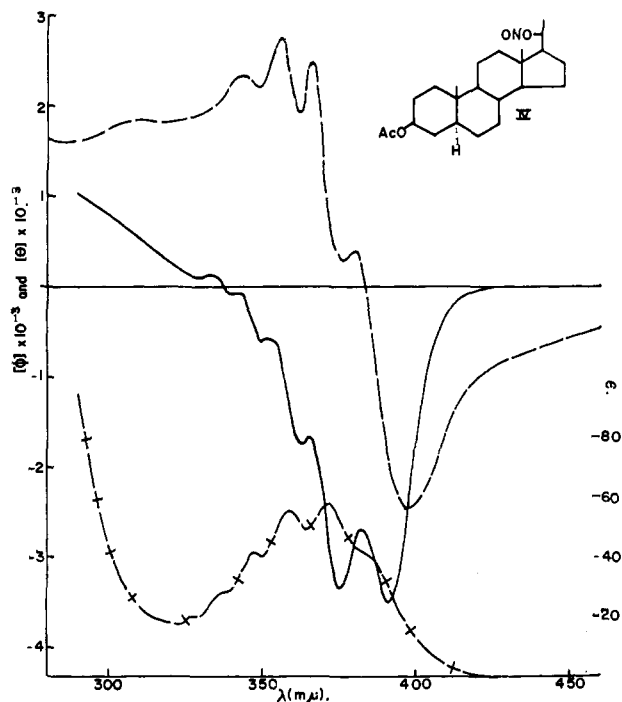


Fig. 4.—Circular dichroism (—), optical rotatory dispersion (---), and ultraviolet absorption (-+ -+) curves of 5α-pregnan-3β,20β-diol 3-acetate 20-nitrite (IV) in dioxane-pyridine (500:1) solution.

370 mμ region and the question arises whether it is possible to determine the stereochemistry at C-20 of this alcohol (VI) through O.R.D. and C.D. measurements of the derived nitrite V, in spite of the presence of the Δ<sup>4</sup>-3-keto chromophore. Inspection of Fig. 5 results in an unambiguously affirmative answer, the strong negative O.R.D. Cotton effect or C.D. negative

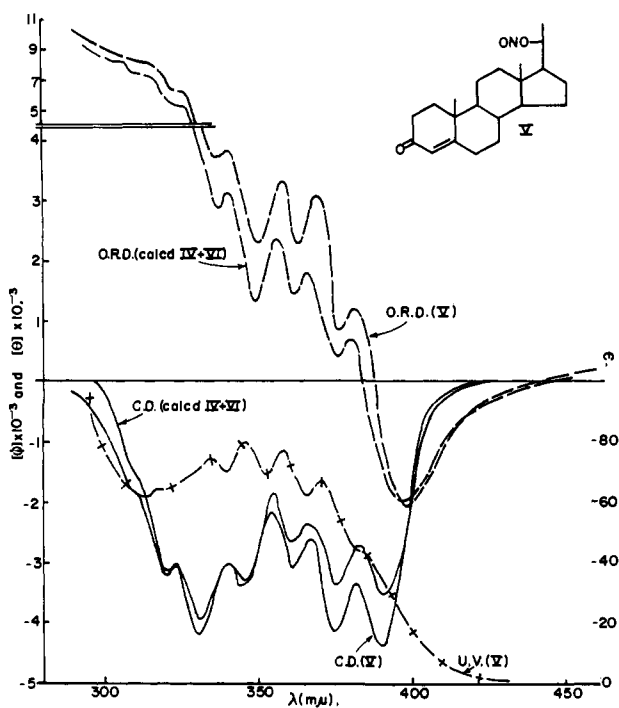


Fig. 5.—Circular dichroism (—), optical rotatory dispersion (-----), and ultraviolet absorption (-+ -+) curves of  $\Delta^4$ -pregnen-20 $\beta$ -ol-3-one 20-nitrite (V) in dioxane-pyridine (500:1) solution.

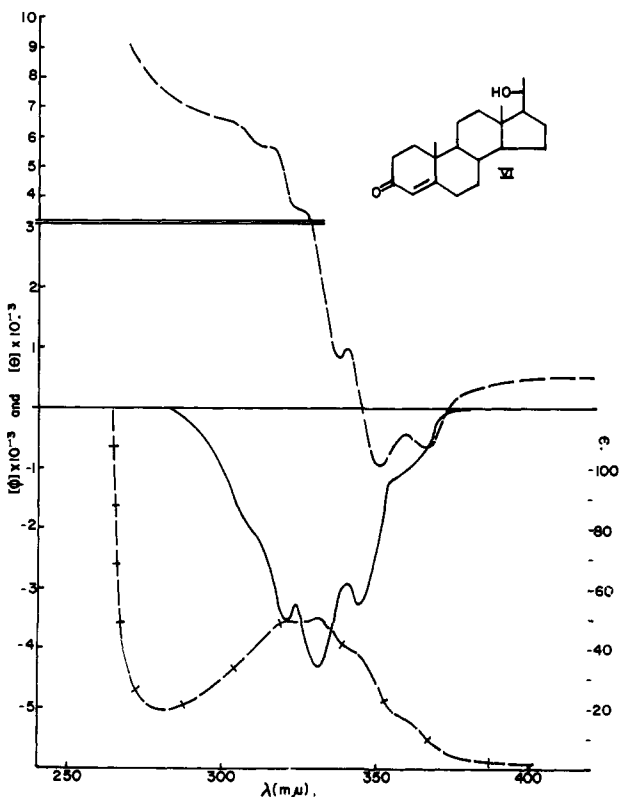


Fig. 6.—Circular dichroism (—), optical rotatory dispersion (-----), and ultraviolet absorption (-+ -+) curves of  $\Delta^4$ -pregnen-20 $\beta$ -ol-3-one (VI) in dioxane solution.

maximum, characteristic (Fig. 4) of the 20 $\beta$ -nitrite grouping, in the 370-400  $m\mu$  region being readily recognizable in Fig. 5. In fact, the contributions of the two chromophores are quite distinct and additive, as shown by a comparison of the calculated O.R.D. and C.D. curves (Fig. 5), composed from the data in Fig.

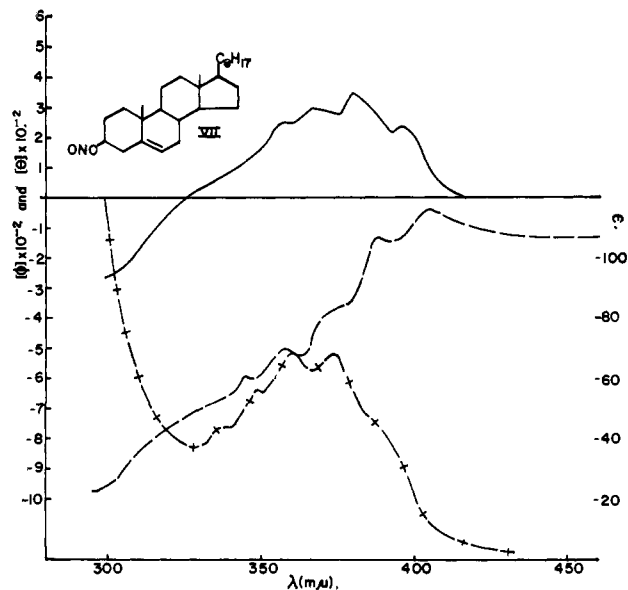


Fig. 7.—Circular dichroism (—), optical rotatory dispersion (-----), and ultraviolet absorption (-+ -+) curves of cholesterol nitrite (VII) in dioxane-pyridine (500:1) solution.

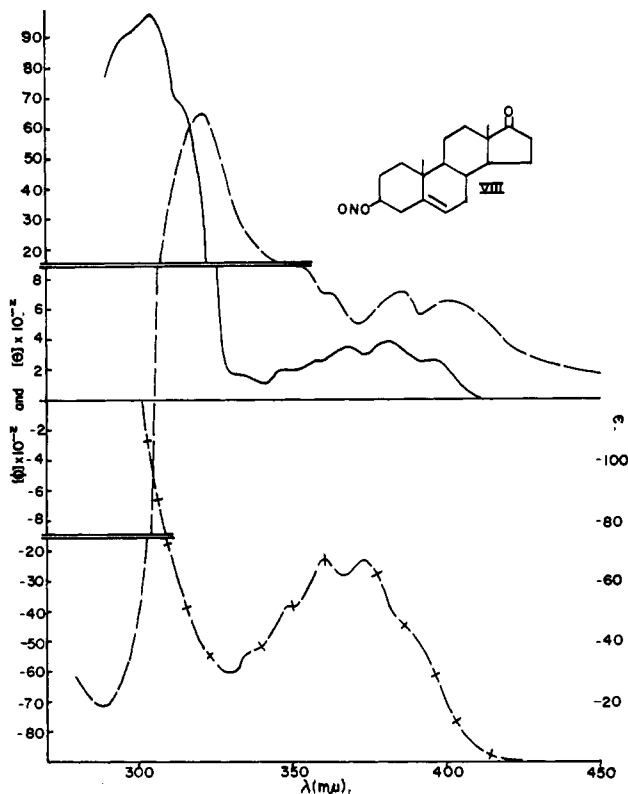


Fig. 8.—Circular dichroism (—), optical rotatory dispersion (-----), and ultraviolet absorption (-+ -+) curves of  $\Delta^5$ -androsten-3 $\beta$ -ol-17-one nitrite (VIII) in dioxane-pyridine (500:1) solution.

4 and 6, and those observed with the unsaturated keto nitrite V.

Since a  $\Delta^5$ -3 $\beta$ -hydroxy nitrite had not been examined earlier,<sup>11</sup> we record in Fig. 7 the pertinent O.R.D., C.D., and ultraviolet properties of a typical representative, cholesterol nitrite (VII). Just as with saturated steroidal 3-nitrites (Fig. 1 and 2 and other examples in ref. 11), the amplitude of the C.D. and O.R.D. curves is quite weak, but the wave length correspondence between the various extrema is excellent. The negative

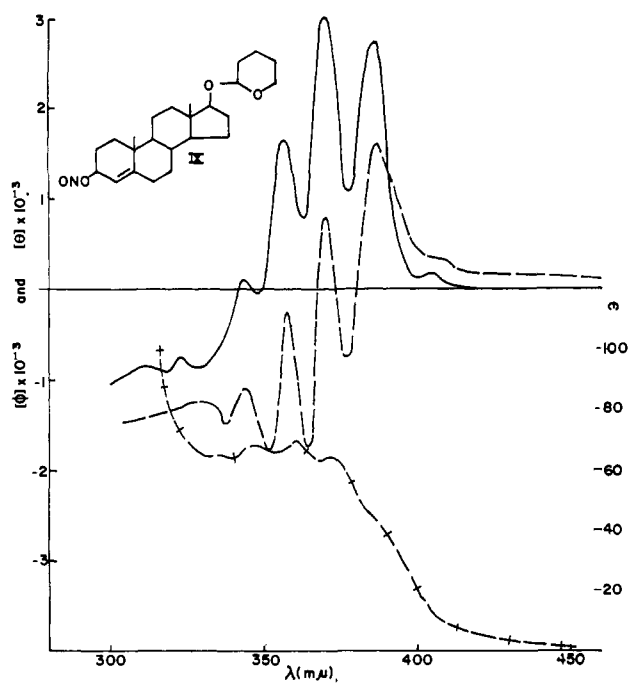


Fig. 9.—Circular dichroism (——), optical rotatory dispersion (-----), and ultraviolet absorption (-+--+ of  $\Delta^4$ -androstene- $3\beta,17\beta$ -diol 3-nitrite 17-tetrahydropyranyl ether (IX) in dioxane-pyridine (500:1) solution.

background rotation associated<sup>15</sup> with the  $\Delta^3$ -double bond is responsible for the fact that the series of positive Cotton effects, characteristic (see Fig. 1) of the saturated  $3\beta$ -nitrite grouping, is now displaced toward negative values. This, of course, does not apply to the C.D. curve, which is not subject to such background effects, the advantages and disadvantages of which have already been commented upon.<sup>3</sup>

It was of interest to see whether the relatively weak rotational strength of the saturated  $3\beta$ -nitrite grouping could be detected in a substance containing a second chromophore. Using  $\Delta^5$ -androstene- $3\beta$ -ol-17-one nitrite (VIII) as an example, where the additional 17-keto group is known<sup>16</sup> to exhibit a positive O.R.D. Cotton effect of substantial amplitude, it can be seen from Fig. 8 that both the O.R.D. and C.D. curves exhibit the multiple positive Cotton effect and C.D. curve of the nitrite in the 350–400  $m\mu$  region, as well as the much stronger positive Cotton effect of the 17-keto group below 320  $m\mu$ .

While the homo-allylic  $\Delta^5$ -double bond had no important effect on the sign or amplitude of the O.R.D. Cotton effect or the (C.D.) molecular ellipticity of the  $3\beta$ -nitrite function (Fig. 1 vs. Fig. 7), the introduction of an allylic double bond as in  $\Delta^4$ -androstene- $3\beta,17\beta$ -diol 3-nitrite 17-tetrahydropyranyl ether (IX) greatly increased the amplitudes (Fig. 9) of the O.R.D. and C.D. curves, without, however, affecting the characteristic positive sign associated with the  $3\beta$ -nitrite function. When the corresponding  $3\beta,17\beta$ -dinitrite X was examined (Fig. 10), the sign of the multiple O.R.D. Cotton effect and C.D. curves became inverted, indicating that the 17-nitrite grouping is characterized by a much more powerful negative Cotton effect and C.D. curve.<sup>17</sup>

In summary, it can be stated that our earlier conclusion<sup>11</sup> about the utility of the nitrite chromophore for

(15) C. Djerassi, W. Closson, and A. E. Lippman, *J. Am. Chem. Soc.*, **78**, 3163 (1956).

(16) C. Djerassi, R. Riniker, and B. Riniker, *ibid.*, **78**, 6362 (1956); W. Klyne, *Tetrahedron*, **13**, 30 (1957).

(17) This latter conclusion has also been reached by Legrand and Viennet (ref. 12) from a study of testosterone nitrite.

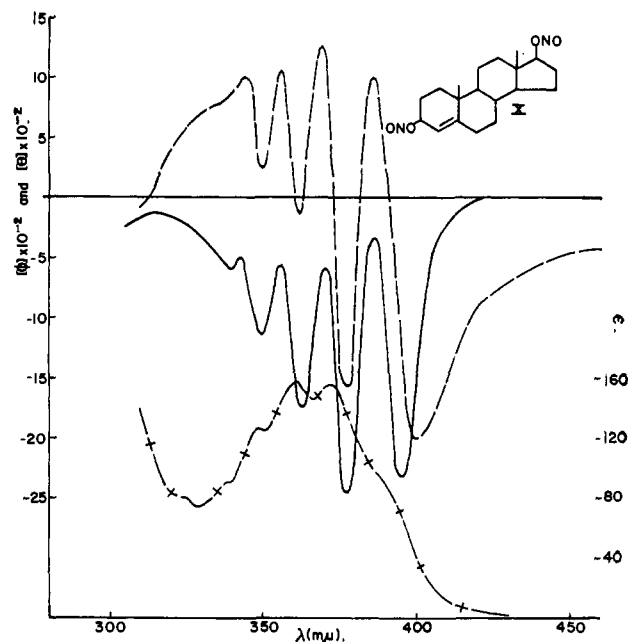


Fig. 10.—Circular dichroism (——), optical rotatory dispersion (-----), and ultraviolet absorption (-+--+ of  $\Delta^4$ -androstene- $3\beta,17\beta$ -diol dinitrite (X) in dioxane-pyridine (500:1) solution.

stereochemical purposes is fully justified and that on the whole, either optical rotatory dispersion or circular dichroism can be utilized interchangeably for this purpose, additional optically active chromophores, such as saturated or unsaturated carbonyl groups, offering no complications. In nitrites of low rotational strength, the O.R.D. background effects (Fig. 1 vs. Fig. 7) are eliminated in C.D. measurements, but, on the other hand, fine structure may at times be recognized more easily (e.g., Fig. 2) by O.R.D.

### The Nitro Chromophore

Prior to 1960, optical rotatory dispersion measurements through the region of the nitro absorption band had been conducted on only six substances, namely, dinitrocamphane<sup>18a</sup> and two related terpenes,<sup>18b</sup> (+)-2-nitrooctane,<sup>19</sup> and two steroidal nitroolefins<sup>19</sup> XIV and XVIII), and the situation was summarized<sup>19</sup> as follows: "These preliminary results indicate that a more extensive rotatory dispersion investigation with parallel spectral studies of various organic nitro compounds should be of considerable interest." Such parallel O.R.D., C.D., and ultraviolet spectral studies have recently been reported<sup>5,20</sup> for aromatic nitro compounds of the bridged and unbridged biphenyl series, where the relative advantages of O.R.D. and C.D. were contrasted.<sup>5</sup> We now report the results of a similar investigation of aliphatic (steroidal) nitro compounds.<sup>21</sup>

An excellent model of an aliphatic nitro derivative is 17 $\beta$ -nitro- $3\beta$ -acetoxy- $5\beta$ -androstane (XI),<sup>22</sup> whose O.R.D., C.D., and ultraviolet spectral properties in methanol are summarized in Fig. 11, where it will be noted that the well-known<sup>23</sup> nitro absorption band in

(18) (a) S. Mitchell and R. R. Gordon, *J. Chem. Soc.*, 853 (1936); the circular dichroism of dinitrocamphane was also measured. (b) G. Jacob, G. Ourisson, and A. Rassat, *Bull. soc. chim. France*, 1374 (1959).

(19) See pp. 194–195 in ref. 7 for otherwise unpublished work from this Laboratory.

(20) K. Mislow, M. A. W. Glass, R. E. O'Brien, P. Rutkin, D. H. Steinberg, J. Weiss, and C. Djerassi, *J. Am. Chem. Soc.*, **84**, 1455 (1962).

(21) Prof. G. Ourisson of the University of Strasbourg and Dr. G. Snatzke of the University of Bonn have informed us that circular dichroism measurements of several nitro compounds have also been performed recently in their respective laboratories.

(22) L. N. Nysted and R. R. Burtner, *J. Org. Chem.*, **27**, 3175 (1962). We are indebted to Dr. Burtner for this specimen.

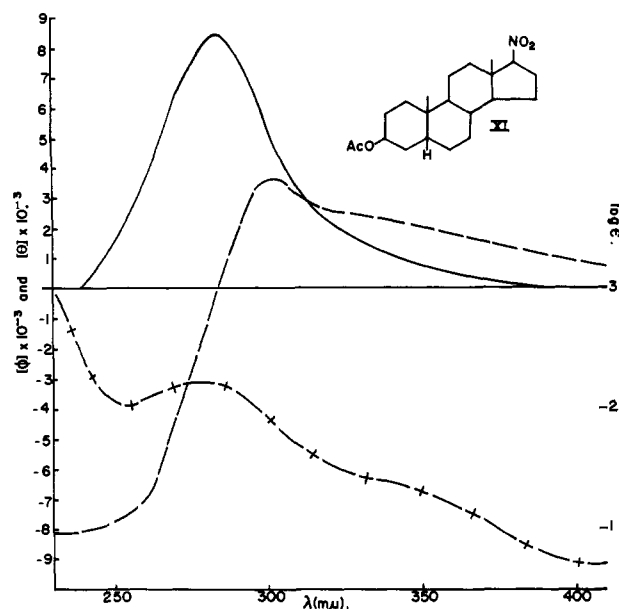


Fig. 11.—Circular dichroism (—), optical rotatory dispersion (---), and ultraviolet absorption (-+ -+) of 17 $\beta$ -nitro-3 $\beta$ -acetoxy-5 $\beta$ -androstane (XI) in methanol solution.

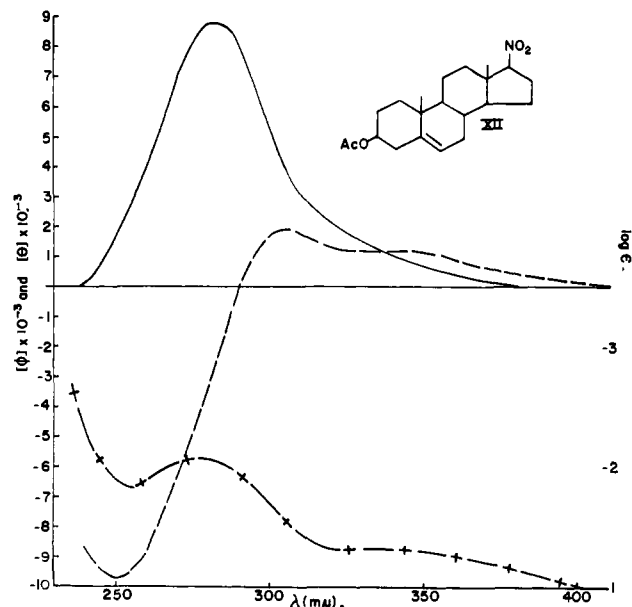


Fig. 12.—Circular dichroism (—), optical rotatory dispersion (---), and ultraviolet absorption (-+ -+) of 17 $\beta$ -nitro-3 $\beta$ -acetoxy- $\Delta^5$ -androstene (XII) in methanol solution.

the 280  $m\mu$  region is optically active. The transition responsible for this absorption has been likened<sup>24</sup> to the carbonyl  $n \rightarrow \pi^*$  transition, but it is pertinent to mention that contrary to the latter's sensitivity toward solvent changes, the positions of the O.R.D., C.D., and ultraviolet curves of the nitro steroid XI in that spectral region were not affected by going from methanol (Fig. 11) to dioxane or isoctane (see Experimental). Of further interest is the existence of a second, long-wave length absorption band—hitherto unreported for nitroalkanes—the presence of which is indicated (Fig. 11) by an inflection in the absorption spectrum near 350  $m\mu$ , an irregularity in the O.R.D. curve and in a tailing

(23) A. Gillam and E. S. Stern, "An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry," Edward Arnold, Ltd., London, 1954, p. 58.

(24) H. H. Jaffé and M. Orchin, "Theory and Applications of Ultraviolet Spectroscopy," John Wiley and Sons, Inc., New York, N. Y., 1962, pp. 182-187.

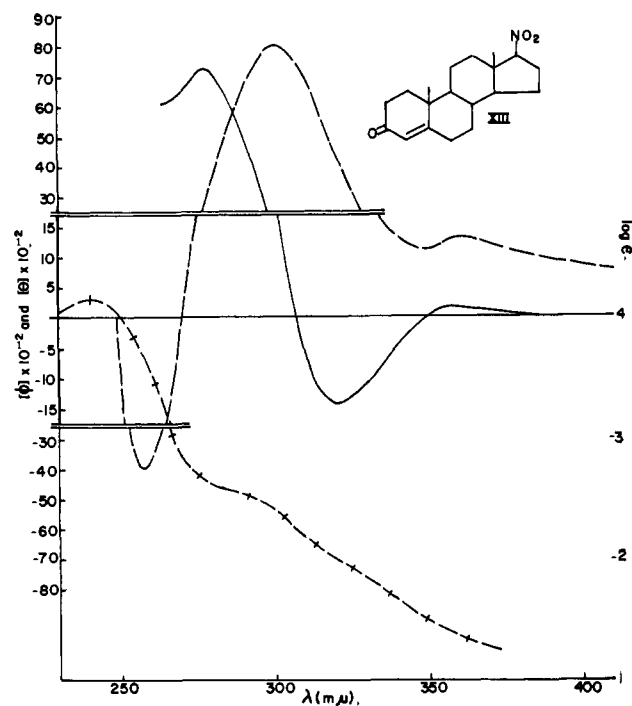


Fig. 13.—Circular dichroism (—), optical rotatory dispersion (---), and ultraviolet absorption (-+ -+) of 17 $\beta$ -nitro- $\Delta^4$ -androsten-3-one (XIII) in methanol solution.

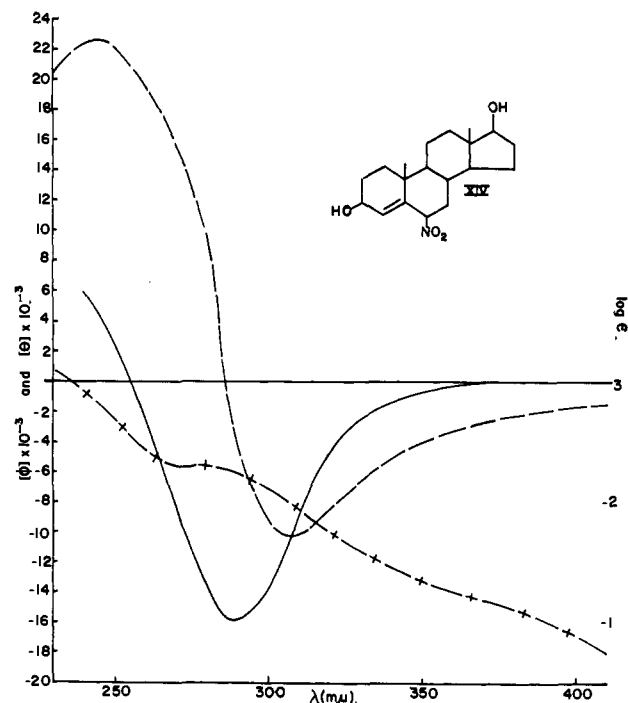


Fig. 14.—Circular dichroism (—), optical rotatory dispersion (---), and ultraviolet absorption (-+ -+) of 6 $\beta$ -nitro- $\Delta^4$ -androstene-3 $\beta$ ,17 $\beta$ -diol (XIV) in methanol solution.

toward long wave length, of the C.D. curve. This second absorption band at longer wave length is even more noticeable (Fig. 12) in 17 $\beta$ -nitro-3 $\beta$ -acetoxy- $\Delta^5$ -androstene (XII),<sup>25</sup> which was kindly provided by Dr. Patchett. It is interesting that in both instances (Fig. 11 and 12), the O.R.D. curve uncovers this absorption band more clearly than the C.D. curve, while more often the reverse is true.

(25) A. A. Patchett, F. Hoffman, F. F. Giarusso, H. Schwam, and G. E. Arth, *J. Org. Chem.*, **27**, 3822 (1962), report the O.R.D. curves (solvent unspecified) of two additional 17 $\beta$ -nitro steroids, where this second O.R.D. extremum near 350  $m\mu$  is also recognizable.

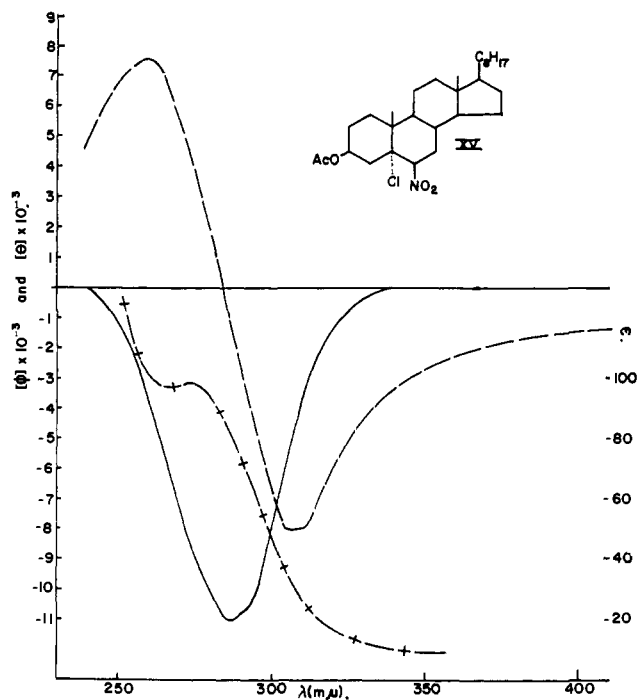


Fig. 15.—Circular dichroism (—), optical rotatory dispersion (-----), and ultraviolet absorption (-+ -+) of  $5\alpha$ -chloro- $6\beta$ -nitrocholestan- $3\beta$ -ol acetate (XV) in methanol solution.

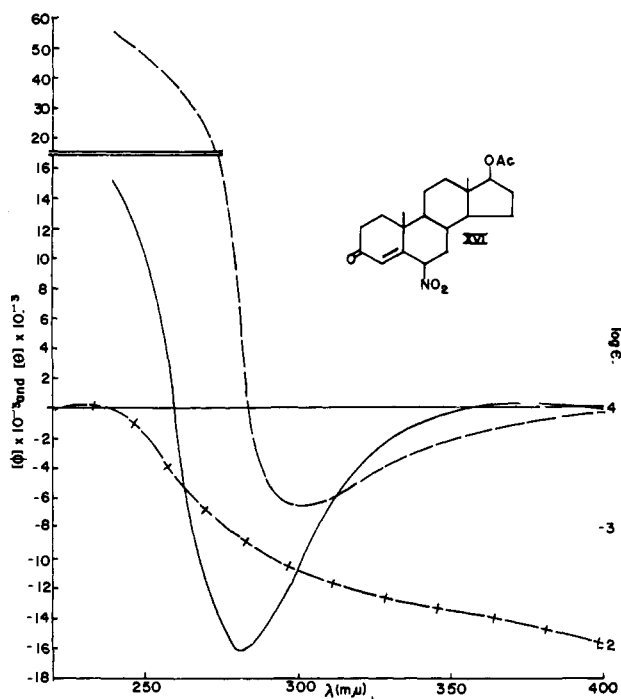


Fig. 16.—Circular dichroism (—), optical rotatory dispersion (-----), and ultraviolet absorption (-+ -+) of  $6\beta$ -nitrotestosterone acetate (XVI) in methanol solution.

An attractive illustration of the utility of O.R.D. and C.D. measurements is afforded by  $17\beta$ -nitro- $\Delta^4$ -androstene- $3$ -one (XIII),<sup>25</sup> where the ultraviolet absorption (Fig. 13) is quite undistinct except for the strong  $239\text{ m}\mu$  absorption of the  $\alpha,\beta$ -unsaturated ketone, the nitro absorption band around  $280\text{ m}\mu$  (see Fig. 11 and 12) appearing only as an inflection, and the  $n \rightarrow \pi^*$  absorption of the  $\alpha,\beta$ -unsaturated ketone above  $300\text{ m}\mu$  being essentially unrecognizable. On the other hand, the negative Cotton effect centered near  $300\text{ m}\mu$  and corresponding to the negative C.D. maximum at that wave

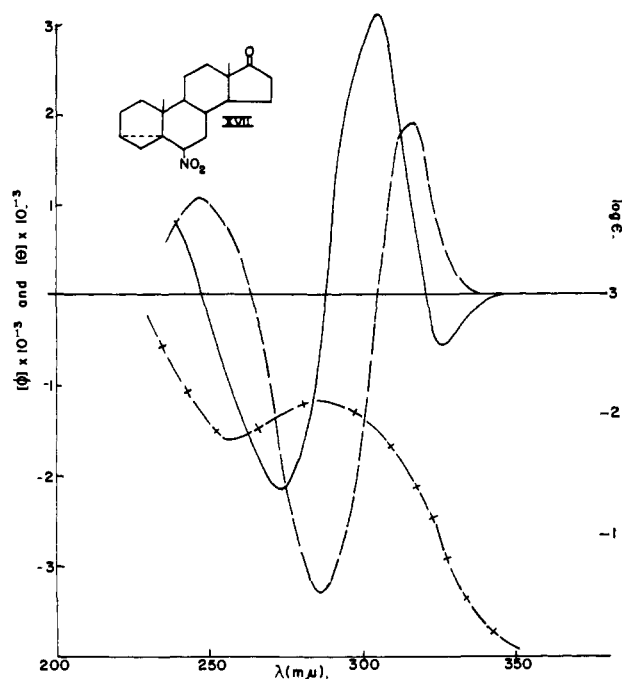


Fig. 17.—Circular dichroism (—), optical rotatory dispersion (-----), and ultraviolet absorption (-+ -+) curves of  $6\beta$ -nitro- $3\alpha,5\alpha$ -cycloandrostan- $17$ -one (XVII) in methanol solution.

length clearly defines the  $n \rightarrow \pi^*$  absorption of the unsaturated carbonyl moiety, while the positive Cotton effect centered at  $278\text{ m}\mu$  and the positive C.D. maximum at  $278\text{ m}\mu$  correspond (see Fig. 11 and 12) to the  $280\text{ m}\mu$  nitro absorption band, which is only ill-defined in the ultraviolet spectrum in Fig. 13. The very small positive C.D. maximum at  $356\text{ m}\mu$  and the corresponding O.R.D. extremum again appear to be associated with the nitro grouping as discussed above in connection with the isolated  $17\beta$ -nitro chromophore (Fig. 11 and 12).

Two nitro steroids,  $6\beta$ -nitro- $\Delta^4$ -androstene- $3\beta,17\beta$ -diol (XIV)<sup>26</sup> and  $5\alpha$ -chloro- $6\beta$ -nitrocholestan- $3\beta$ -ol acetate (XV),<sup>27</sup> which lack additional chromophores, were examined. Both are characterized by strong negative O.R.D. Cotton effects (Fig. 14 and 15) centered in the region of the nitro absorption. The C.D. negative maxima occur at  $288$  and  $287\text{ m}\mu$ , respectively, and are thus displaced somewhat to higher wave length as compared to the ultraviolet absorption maximum.

$6\beta$ -Nitrotestosterone acetate (XVI)<sup>26</sup> represents again a substance with two chromophores, except that in this instance they are sufficiently closely situated to exhibit some interaction. Only the  $\pi \rightarrow \pi^*$  ultraviolet absorption has been recorded<sup>26,28</sup> and the effect of the nitro group noted. As shown in Fig. 16, the  $n \rightarrow \pi^*$  absorption of the  $\alpha,\beta$ -unsaturated ketone is not detectable by any of the three parameters, but the ultraviolet spectrum does not even exhibit any indication of the nitro absorption, while this is clearly recognizable through the negative Cotton effect (trough at  $302\text{ m}\mu$ ) or the negative C.D. maximum ( $281\text{ m}\mu$ ), which are only slightly displaced to shorter wave length as compared to the isolated  $6$ -nitro steroids XIV and XV. An equally

(26) A. Bowers, M. B. Sanchez, and H. J. Ringold, *J. Am. Chem. Soc.*, **81**, 3702 (1959). The O.R.D. curves of a number of  $6\alpha$ - and  $6\beta$ -nitro- $\Delta^4$ - $3$ -keto steroids are reported in this article and the one by A. Bowers, L. C. Ibañez, and H. J. Ringold, *ibid.*, **81**, 3707 (1959). We are indebted to Dr. Bowers (Syntex, S. A., Mexico City) for supplying several of the nitro steroids (XIV, XVI, XV11, XIX).

(27) K. Tanabe and R. Hayashi, *Chem. Pharm. Bull. (Tokyo)*, **10**, 1180 (1962).

(28) H. J. Ringold and A. Bowers, *Experientia*, **17**, 65 (1961).

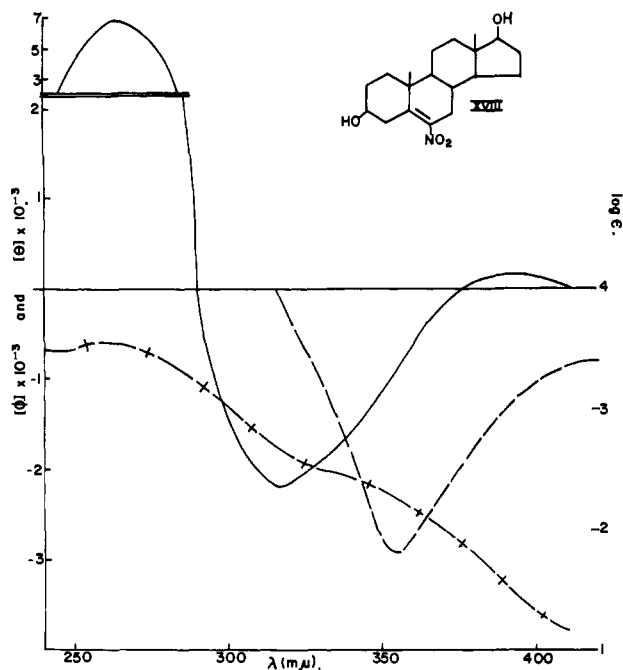


Fig. 18.—Circular dichroism (—), optical rotatory dispersion (-----), and ultraviolet absorption (-+ -+) curves of 6-nitro- $\Delta^5$ -androstene-3 $\beta$ ,17 $\beta$ -diol (XVIII) in methanol solution.

striking example of uncovering of a "hidden" ultraviolet spectral transition is afforded by 6 $\beta$ -nitro-3 $\alpha$ ,5 $\alpha$ -cycloandrostane-17-one (XVII),<sup>29</sup> which contains the 6 $\beta$ -nitro group, absorbing around 280  $m\mu$  and characterized by a negative Cotton effect (see XIV and XV), as well as the saturated 17-keto chromophore with its low-intensity ultraviolet absorption maximum at 292–293  $m\mu$ <sup>30</sup> and a substantial positive Cotton effect.<sup>16</sup> While the ultraviolet absorption spectrum (Fig. 17) of XVII shows only a single maximum at 286  $m\mu$ —approximately half way between the nitro and ketone absorption bands—the O.R.D. and C.D. curves demonstrate conclusively the presence of both chromophores, the long-wave length positive Cotton effect and positive C.D. maximum corresponding to the 17-keto group, while the negative Cotton effect and negative C.D. maximum point unequivocally toward the contribution of the 6 $\beta$ -nitro function.

The optical and spectral data collected in Fig. 18 for the conjugated nitroolefin 6-nitro- $\Delta^5$ -androstene-3 $\beta$ ,17 $\beta$ -diol (XVIII)<sup>26</sup> settle an apparent anomaly noted earlier.<sup>19</sup> At that time, attention was called to the fact that while the  $\Delta^5$ -6-nitro derivative XVIII exhibits an ultraviolet maximum near 260  $m\mu$  (solvent dependent!), its first rotatory dispersion extremum appears at 355  $m\mu$  (see Fig. 18), whereas the nonconjugated double bond isomer XIV shows this extremum near 310  $m\mu$ , but absorbs at higher wave length (*ca.* 280  $m\mu$ ; see Fig. 14). This apparent discrepancy is now resolved by the observation (Fig. 18) that the conjugated isomer XVIII possesses a second ultraviolet absorption band—only indicated by a flat shoulder between 370–330  $m\mu$  (Fig. 18), which is strongly optically active and which gave rise to the earlier noted<sup>19</sup> first extremum of a negative Cotton effect, which now finds its counterpart (Fig. 18) in a negative C.D. maximum at 317  $m\mu$ . Further ultraviolet penetration in terms of O.R.D. was not possible experimentally in this case, but it proved feasible to penetrate into this spectral region spectrodichrometri-

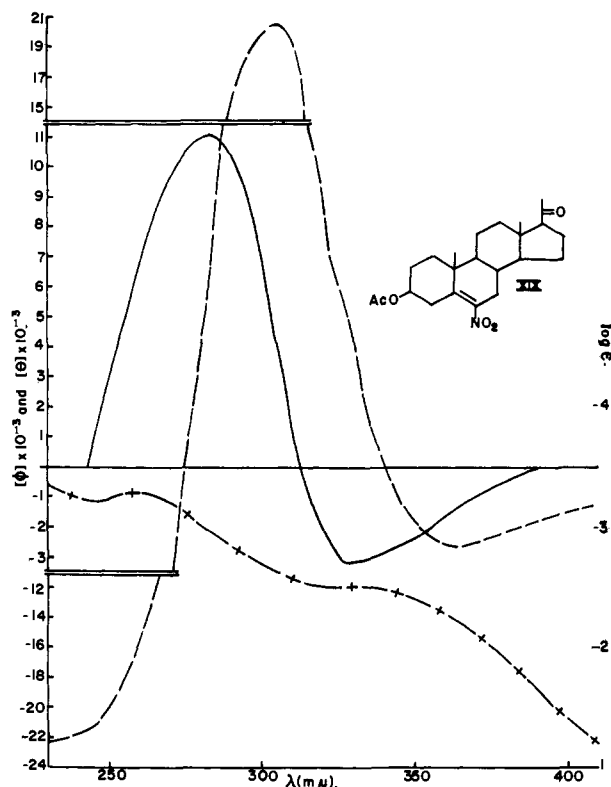


Fig. 19.—Circular dichroism (—), optical rotatory dispersion (-----), and ultraviolet absorption (-+ -+) curves of 6-nitro- $\Delta^5$ -pregnen-3 $\beta$ -ol-20-one acetate (XIX) in methanol solution.

cally, thus demonstrating (Fig. 18) that the 260  $m\mu$  absorption band was also strongly optically active and of opposite sign to the 317  $m\mu$  band. The shape of the O.R.D. curve near 400  $m\mu$  and especially the weak positive C.D. maximum at 395  $m\mu$  again point to the presence of a long-wave length, low-intensity absorption band, evidently resembling that noted in the saturated 17 $\beta$ -nitro curves (Fig. 11 and 12). The situation illustrated by Fig. 18 represents an instance where circular dichroism measurements are to be preferred over O.R.D.

Finally, in Fig. 19, there is collected the relevant information on a conjugated nitroolefin, 6-nitro- $\Delta^5$ -pregnen-3 $\beta$ -ol-20-one acetate (XIX),<sup>26</sup> which also contains the saturated 20-keto group with its powerful positive Cotton effect.<sup>31</sup> The long-wave length band of the conjugated nitro chromophore is recognizable by the shoulder (320–350  $m\mu$ ) in the ultraviolet spectrum (Fig. 19) as well as by the negative Cotton effect and the negative C.D. maximum in that region. The  $n \rightarrow \pi^*$  transition of the 20-ketone, however, is completely obscured in the ultraviolet spectrum by the stronger 258  $m\mu$  absorption maximum of the nitro group. Its presence is nevertheless demonstrated in an unequivocal fashion by the strong positive Cotton effect in the expected region<sup>31</sup> and the corresponding positive C.D. maximum (Fig. 19).

In conclusion, the present O.R.D. and C.D. measurements with various nitro chromophores have shed considerable light on a number of ultraviolet spectral transitions and they have again demonstrated the power of these two phenomenologically related methods for uncovering hidden or overlapping absorption bands, which are not readily discernible by the standard ultraviolet spectral techniques.

(29) J. Tadanier and W. Cole, *J. Org. Chem.*, **27**, 4615 (1962). We are indebted to these authors for freshly prepared samples of VIII and XVII.

(30) C. W. Shoppee, R. H. Jenkins, and G. H. R. Summers, *J. Chem. Soc.*, 3048 (1958); J. Fishman and C. Djerassi, *Experientia*, **16**, 138 (1960).

(31) C. Djerassi, O. Halpern, V. Halpern, O. Schindler, and C. Tamm, *Helv. Chim. Acta*, **41**, 250 (1958).

Experimental<sup>32-34</sup>

**Cholestan-3 $\beta$ -ol nitrite (I)** (Fig. 1), m.p. 110° (from methanol): Anal. Calcd. for  $C_{27}H_{47}NO_2$ : N, 3.36. Found: N, 3.45. C.D. (c 2.315, 440–320  $\mu$ m):  $[\theta]_{440} 0$ ,  $[\theta]_{386} +490$ ,  $[\theta]_{377} +350$ ,  $[\theta]_{370} +490$ ,  $[\theta]_{362} +300$ ,  $[\theta]_{357} +370$ ,  $[\theta]_{349} +185$ ,  $[\theta]_{347} +210$ ,  $[\theta]_{323} 0$ . O.R.D. (c 0.1160, 460–310  $\mu$ m):  $[\phi]_{392} +600^\circ$ ,  $[\phi]_{383} +275^\circ$ ,  $[\phi]_{377} +400^\circ$ ,  $[\phi]_{368} +30^\circ$ ,  $[\phi]_{362} +125^\circ$ ,  $[\phi]_{355} -50^\circ$ ,  $[\phi]_{350} +45^\circ$ ,  $[\phi]_{345} 0^\circ$ ,  $[\phi]_{340} +60^\circ$ ,  $[\phi]_{337} +45^\circ$ . Ultraviolet spectrum:  $\lambda_{340-356}^{shoulder} \in 36$ ,  $\lambda_{330-324}^{shoulder} \in 27$ .

**Cholestan-3 $\alpha$ -ol nitrite (II)** (Fig. 2), m.p. 104° (from hexane): Anal. Calcd. for  $C_{27}H_{47}NO_2$ : N, 3.36. Found: N, 3.14. C.D. (c 3.965, 410–300  $\mu$ m):  $[\theta]_{405} 0$ ,  $[\theta]_{391} -120$ ,  $[\theta]_{382} -90$ ,  $[\theta]_{371} -130$ ,  $[\theta]_{360} -80$ ,  $[\theta]_{358} -105$ ,  $[\theta]_{346} -25$ ,  $[\theta]_{333} 0$ ,  $[\theta]_{300} +65$ . O.R.D. (c 0.209, 460–370  $\mu$ m); c 0.1045, 370–320  $\mu$ m):  $[\phi]_{355} +130^\circ$ ,  $[\phi]_{350} +80^\circ$ ,  $[\phi]_{346} +205^\circ$ ,  $[\phi]_{340} +135^\circ$ ,  $[\phi]_{331} +290^\circ$ ,  $[\phi]_{335} +240^\circ$ ,  $[\phi]_{346} +325^\circ$ ,  $[\phi]_{339} +255^\circ$ ,  $[\phi]_{320} +390^\circ$ . Ultraviolet spectrum:  $\lambda_{395-355}^{shoulder} \in 31-42$ ,  $\lambda_{373}^{max} \in 64$ ,  $\lambda_{360}^{max} \in 65$ ,  $\lambda_{345}^{max} \in 50$ ,  $\lambda_{340-356}^{shoulder} \in 36$ ,  $\lambda_{330-324}^{shoulder} \in 27$ .

**5 $\alpha$ -Pregnan-20 $\alpha$ -ol nitrite (III)** (Fig. 3), m.p. 88–89° (from hexane): Anal. Calcd. for  $C_{27}H_{47}NO_2$ : N, 4.20. Found: N, 4.16. C.D. (c 3.073, 430–290  $\mu$ m):  $[\theta]_{430} 0$ ,  $[\theta]_{392} +2700$ ,  $[\theta]_{382} +1820$ ,  $[\theta]_{375} +2430$ ,  $[\theta]_{367} +855$ ,  $[\theta]_{362} +1020$ ,  $[\theta]_{353} -40$ ,  $[\theta]_{349} +85$ ,  $[\theta]_{343} -385$ ,  $[\theta]_{338} -235$ ,  $[\theta]_{332} -300$ ,  $[\theta]_{328-322} -235$ ,  $[\theta]_{290} -85$ . O.R.D. (c 0.127, 460–300  $\mu$ m):  $[\phi]_{400} +2180^\circ$ ,  $[\phi]_{382} -430^\circ$ ,  $[\phi]_{376} -145^\circ$ ,  $[\phi]_{368} -2130^\circ$ ,  $[\phi]_{363} -960^\circ$ ,  $[\phi]_{357} -2100^\circ$ ,  $[\phi]_{350} -1010^\circ$ ,  $[\phi]_{343} -1300^\circ$ ,  $[\phi]_{337} -675^\circ$ ,  $[\phi]_{333} -755^\circ$ ,  $[\phi]_{311} -400^\circ$ . Ultraviolet spectrum:  $\lambda_{395-350}^{shoulder} \in 42$ ,  $\lambda_{371}^{max} \in 63$ ,  $\lambda_{358}^{max} \in 60$ ,  $\lambda_{346}^{max} \in 46$ ,  $\lambda_{330-334}^{shoulder} \in 33-31$ ,  $\lambda_{329-324}^{shoulder} \in 24$ .

**5 $\alpha$ -Pregnan-3 $\beta$ ,20 $\beta$ -diol 3-acetate 20-nitrite (IV)** (Fig. 4), m.p. 154–155° (from methylene dichloride-hexane): Anal. Calcd. for  $C_{27}H_{47}NO_4$ : N, 3.58. Found: N, 3.55. C.D. (c 2.81, 430–290  $\mu$ m):  $[\theta]_{430} 0$ ,  $[\theta]_{391} -3520$ ,  $[\theta]_{382} -2700$ ,  $[\theta]_{375} -3370$ ,  $[\theta]_{365} -1670$ ,  $[\theta]_{362} -1740$ ,  $[\theta]_{351} -570$ ,  $[\theta]_{350} -600$ ,  $[\theta]_{343-339} -70$ ,  $[\theta]_{333} +140$ ,  $[\theta]_{329} +105$ ,  $[\theta]_{290} +1060$ . O.R.D. (c 0.2355, 460–280  $\mu$ m):  $[\phi]_{397} -2460^\circ$ ,  $[\phi]_{380} +400^\circ$ ,  $[\phi]_{376} +265^\circ$ ,  $[\phi]_{366} +2460^\circ$ ,  $[\phi]_{362} +1930^\circ$ ,  $[\phi]_{356} +2730^\circ$ ,  $[\phi]_{349} +2200^\circ$ ,  $[\phi]_{243} +2330^\circ$ ,  $[\phi]_{325-308} +1860^\circ$ ,  $[\phi]_{290} +1600^\circ$ ,  $[\phi]_{280} +1670^\circ$ . Ultraviolet spectrum:  $\lambda_{390-350}^{shoulder} \in 31-43$ ,  $\lambda_{371}^{max} \in 58$ ,  $\lambda_{358}^{max} \in 55$ ,  $\lambda_{346}^{max} \in 42$ ,  $\lambda_{330-306}^{shoulder} \in 28$ ,  $\lambda_{329-323}^{shoulder} \in 20$ .

**$\Delta^1$ -Pregnen-20 $\beta$ -ol-3-one 20-nitrite (V)** (Fig. 5), m.p. 156° (from methanol): Anal. Calcd. for  $C_{27}H_{45}NO_3$ : N, 4.05. Found: N, 3.97. C.D. (c 1.78, 430–290  $\mu$ m):  $[\theta]_{430} 0$ ,  $[\theta]_{390} -4380$ ,  $[\theta]_{382} -3370$ ,  $[\theta]_{375} -4160$ ,  $[\theta]_{367} -2620$ ,  $[\theta]_{361} -3070$ ,  $[\theta]_{354} -2180$ ,  $[\theta]_{346} -3320$ ,  $[\theta]_{340} -3020$ ,  $[\theta]_{331} -3960$ ,  $[\theta]_{323} -3020$ ,  $[\theta]_{320} -3140$ ,  $[\theta]_{308} -1660$ ,  $[\theta]_{290} -200$ . O.R.D. (c 1.36, 460–290  $\mu$ m):  $[\phi]_{398} -2090^\circ$ ,  $[\phi]_{381} -1170^\circ$ ,  $[\phi]_{376} +860^\circ$ ,  $[\phi]_{369} +3060^\circ$ ,  $[\phi]_{363} +2290^\circ$ ,  $[\phi]_{358} +3310^\circ$ ,  $[\phi]_{350} +2290^\circ$ ,  $[\phi]_{346} +3820^\circ$ ,  $[\phi]_{336} +3720^\circ$ ,  $[\phi]_{325} +6260^\circ$ ,  $[\phi]_{313} +8140^\circ$ . Ultraviolet spectrum:  $\lambda_{390-380}^{shoulder} \in 35-47$ ,  $\lambda_{371}^{max} \in 67$ ,  $\lambda_{358}^{max} \in 77$ ,  $\lambda_{346}^{max} \in 80$ ,  $\lambda_{334}^{max} \in 74$ ,  $\lambda_{320-325}^{shoulder} \in 64-63$ .

**$\Delta^1$ -Pregnen-20 $\beta$ -ol-3-one (VI)**<sup>35</sup> (Fig. 6): C.D. in dioxane (c 1.056 390–280  $\mu$ m):  $[\theta]_{380} 0$ ,  $[\theta]_{354} -1180$ ,  $[\theta]_{344} -3280$ ,  $[\theta]_{339} -2930$ ,  $[\theta]_{331} -4300$ ,  $[\theta]_{323} -3240$ ,  $[\theta]_{320} -3500$ ,  $[\theta]_{305} -1680$ ,  $[\theta]_{280} 0$ . O.R.D. in dioxane (c 0.133, 420–270  $\mu$ m):  $[\phi]_{365} -615^\circ$ ,  $[\phi]_{359} -430^\circ$ ,  $[\phi]_{350} -920^\circ$ ,  $[\phi]_{340} +985^\circ$ ,  $[\phi]_{337} +860^\circ$ ,  $[\phi]_{327} +3440^\circ$ ,  $[\phi]_{323} +3560^\circ$ ,  $[\phi]_{316-312} +5660^\circ$ . Ultraviolet spectrum in dioxane:  $\lambda_{365-345}^{shoulder} \in 12-20$ ,  $\lambda_{350-340}^{shoulder} \in 30-41$ ,  $\lambda_{330}^{max} \in 50$ ,  $\lambda_{325-319}^{shoulder} \in 48$ .

(32) All optical rotatory dispersion and circular dichroism data are reported in the manner outlined in footnote 28 of ref. 3. The following instruments were employed: Japan Spectroscopic Manufacturing Co. (Nippon Bunko) automatically recording spectropolarimeter, Baird-Atomic-Jouan Dichrograph, and Cary Applied Physics Model 14 ultraviolet spectrophotometer.

(33) All nitrites were prepared by the following procedure, which is essentially that of D. H. R. Barton, J. M. Beaton, L. E. Geller, and M. M. Pechet (*J. Am. Chem. Soc.*, **83**, 4076 (1961)), additional helpful advice having been provided by Dr. A. L. Nussbaum (Department of Biochemistry, Stanford University Medical School). The steroid alcohol (200 mg.) was dissolved in 5 cc. of dry pyridine and cooled in an ice bath to 0°. An excess of gaseous nitrosyl chloride was passed very slowly into the stirred solution until a slight red-brown color persisted, whereupon stirring at 0° was continued for an additional 10 min. After dilution with 100 cc. of ice-water, the resulting solid was collected, washed with water, dried *in vacuo* at room temperature for 6 hr., and then recrystallized from the indicated solvent by dissolving at room temperature and concentrating the solution *in vacuo* without heat. The homogeneity of each specimen was tested by thin-layer chromatography on silica gel with benzene-methanol (9:1), the spots being detected by spraying with ceric sulfate solution. All optical and spectral measurements of such freshly prepared nitrites were performed in dioxane solutions containing 0.2% pyridine, since the absence of such a stabilizer resulted in gradually reduced intensity values.

(34) For circular dichroism nomenclature, see C. Djerassi and E. Bunnenberg, *Proc. Chem. Soc.*, in press.

(35) P. Wieland and K. Miessner, *Helv. Chim. Acta*, **32**, 1922 (1949); J. Romo, M. Romero, C. Djerassi, and G. Rosenkranz, *J. Am. Chem. Soc.*, **73**, 1528 (1951).

**Cholesterol nitrite (VII)** (Fig. 7), m.p. 89–91° (from hexane): Anal. Calcd. for  $C_{27}H_{45}NO_2$ : N, 3.37. Found: N, 3.41. C.D. (c 3.84, 420–300  $\mu$ m):  $[\theta]_{415} 0$ ,  $[\theta]_{395} +235$ ,  $[\theta]_{392} +220$ ,  $[\theta]_{380} +350$ ,  $[\theta]_{375} +280$ ,  $[\theta]_{368} +305$ ,  $[\theta]_{360} +250$ ,  $[\theta]_{356} +250$ ,  $[\theta]_{326} 0$ ,  $[\theta]_{300} -265$ . O.R.D. (c 0.1403, 460–295  $\mu$ m):  $[\phi]_{450} -135^\circ$ ,  $[\phi]_{405} -40^\circ$ ,  $[\phi]_{392} -145^\circ$ ,  $[\phi]_{388} -130^\circ$ ,  $[\phi]_{370} -395^\circ$ ,  $[\phi]_{368} -525^\circ$ ,  $[\phi]_{358} -505^\circ$ ,  $[\phi]_{347} -605^\circ$ ,  $[\phi]_{344} -590^\circ$ ,  $[\phi]_{295} -975^\circ$ . Ultraviolet spectrum:  $\lambda_{385-385}^{shoulder} \in 34-47$ ,  $\lambda_{375}^{max} \in 68$ ,  $\lambda_{370}^{max} \in 68$ ,  $\lambda_{347}^{max} \in 57$ ,  $\lambda_{340-336}^{shoulder} \in 44$ .

**$\Delta^5$ -Androsten-3 $\beta$ -ol-17-one nitrite (VIII)**<sup>29</sup> (Fig. 8): C.D. (c 2.313, 420–330  $\mu$ m); c 0.4626, 330–290  $\mu$ m):  $[\theta]_{413} 0$ ,  $[\theta]_{396} +260$ ,  $[\theta]_{391} +230$ ,  $[\theta]_{381} +385$ ,  $[\theta]_{373} +295$ ,  $[\theta]_{367} +350$ ,  $[\theta]_{360-356} +245$ ,  $[\theta]_{350-345} +195$ ,  $[\theta]_{341} +105$ ,  $[\theta]_{334} +6740$ ,  $[\theta]_{304} +9710$ ,  $[\theta]_{290} +7800$ . O.R.D. (c 0.177, 450–315  $\mu$ m); c 0.0885, 315–300  $\mu$ m); c 0.0221, 300–280  $\mu$ m):  $[\phi]_{402} +660^\circ$ ,  $[\phi]_{391} +575^\circ$ ,  $[\phi]_{385} +715$ ,  $[\phi]_{371} +500^\circ$ ,  $[\phi]_{362-360} +720^\circ$ ,  $[\phi]_{354} +1100^\circ$ ,  $[\phi]_{321} +6450^\circ$ ,  $[\phi]_{288} -7160^\circ$ . Ultraviolet spectrum:  $\lambda_{395-385}^{shoulder} \in 32-46$ ,  $\lambda_{375}^{max} \in 68$ ,  $\lambda_{360}^{max} \in 68$ ,  $\lambda_{345}^{max} \in 52$ ,  $\lambda_{340-336}^{shoulder} \in 38-37$ .

**$\Delta^1$ -Androstene-3 $\beta$ ,17 $\beta$ -diol 3-Nitrite 17-Tetrahydropyranyl Ether (IX)** (Fig. 9).—Testosterone tetrahydropyranyl ether<sup>36</sup> was reduced with lithium aluminum hydride in ether solution (20 min., room temperature) and the resulting  $\Delta^1$ -androsten-3 $\beta$ ,17 $\beta$ -diol 17-tetrahydropyranyl ether purified by alumina chromatography and recrystallization from hexane; m.p. 129–130°,  $\alpha_D^{CHCl_3} +27^\circ$ .

Anal. Calcd. for  $C_{27}H_{48}O_3$ : C, 76.96; H, 10.23. Found: C, 76.70; H, 10.21.

The alcohol was then transformed by the standard technique<sup>33</sup> into the nitrite IX, m.p. 83–85° (from hexane): Anal. Calcd. for  $C_{27}H_{47}NO_4$ : N, 3.48. Found: N, 3.27. C.D. (c 1.68, 420–300  $\mu$ m):  $[\theta]_{420} 0$ ,  $[\theta]_{405} +185$ ,  $[\theta]_{400} +125$ ,  $[\theta]_{386} +2760$ ,  $[\theta]_{377} +1105$ ,  $[\theta]_{370} +3015$ ,  $[\theta]_{363} +800$ ,  $[\theta]_{357} +1660$ ,  $[\theta]_{348} -60$ ,  $[\theta]_{343} +125$ ,  $[\theta]_{330-328} -860$ ,  $[\theta]_{323} -740$ ,  $[\theta]_{318} -920$ ,  $[\theta]_{312} -830$ ,  $[\theta]_{300} -1045$ . O.R.D. (c 0.0927, 460–300  $\mu$ m):  $[\phi]_{408-402} +330^\circ$ ,  $[\phi]_{387} +1610^\circ$ ,  $[\phi]_{377} -740^\circ$ ,  $[\phi]_{370} +870^\circ$ ,  $[\phi]_{364} -1740^\circ$ ,  $[\phi]_{358} -260^\circ$ ,  $[\phi]_{351} -1780^\circ$ ,  $[\phi]_{344} -1085^\circ$ ,  $[\phi]_{338} -1480^\circ$ ,  $[\phi]_{330} -1300^\circ$ ,  $[\phi]_{300} -1520^\circ$ . Ultraviolet spectrum:  $\lambda_{390-386}^{shoulder} \in 38-53$ ,  $\lambda_{371}^{max} \in 64$ ,  $\lambda_{360}^{max} \in 70$ ,  $\lambda_{347}^{max} \in 68$ ,  $\lambda_{336-332}^{shoulder} \in 65$ .

**$\Delta^1$ -Androstene-3 $\beta$ ,17 $\beta$ -diol dinitrite (X)** (Fig. 10), m.p. 106–107° (from hexane): Anal. Calcd. for  $C_{27}H_{47}N_2O_4$ : N, 8.06. Found: N, 7.72. C.D. (c 1.333, 420–305  $\mu$ m):  $[\theta]_{420} 0$ ,  $[\theta]_{395} -2320$ ,  $[\theta]_{386} -330$ ,  $[\theta]_{377} -2460$ ,  $[\theta]_{370} -600$ ,  $[\theta]_{362} -1730$ ,  $[\theta]_{356} -565$ ,  $[\theta]_{349} -1130$ ,  $[\theta]_{342} -500$ ,  $[\theta]_{338} -565$ ,  $[\theta]_{315} -135$ ,  $[\theta]_{305} -230$ . O.R.D. (c 0.115, 460–310  $\mu$ m):  $[\phi]_{400} -2000^\circ$ ,  $[\phi]_{386} +1000^\circ$ ,  $[\phi]_{377} -1560^\circ$ ,  $[\phi]_{369} +1250^\circ$ ,  $[\phi]_{362} -125^\circ$ ,  $[\phi]_{356} +1600^\circ$ ,  $[\phi]_{350} +250^\circ$ ,  $[\phi]_{344} +1000^\circ$ ,  $[\phi]_{310} -100^\circ$ . Ultraviolet spectrum:  $\lambda_{385-385}^{shoulder} \in 70-102$ ,  $\lambda_{371}^{max} \in 156$ ,  $\lambda_{360}^{max} \in 154$ ,  $\lambda_{348}^{max} \in 127$ ,  $\lambda_{339-337}^{shoulder} \in 90$ ,  $\lambda_{326-320}^{shoulder} \in 77-82$ .

**17 $\beta$ -Nitro-3 $\beta$ -acetoxy-5 $\beta$ -androstene (XI)**<sup>22</sup> (Fig. 11): C.D. in methanol (c 0.888, 390–250  $\mu$ m); c 0.1777, 250–240  $\mu$ m):  $[\theta]_{390} 0$ ,  $[\theta]_{283} +8400$ ,  $[\theta]_{240} 0$ . C.D. in dioxane (c 0.60, 380–260  $\mu$ m); c 0.012, 260–250  $\mu$ m):  $[\theta]_{380} 0$ ,  $[\theta]_{281} +8080$ ,  $[\theta]_{250} +2250$ . C.D. in isoctane (c 0.485, 380–260  $\mu$ m); c 0.097, 260–245  $\mu$ m):  $[\theta]_{380} 0$ ,  $[\theta]_{284} +7400$ ,  $[\theta]_{245} 0$ . O.R.D. in methanol (c 0.0888, 410–280  $\mu$ m); c 0.0035, 280–230  $\mu$ m):  $[\phi]_{303} +3630^\circ$ . O.R.D. in dioxane (c 0.097, 420–290  $\mu$ m); c 0.0194, 290–240  $\mu$ m):  $[\phi]_{356-340} +1190^\circ$ ,  $[\phi]_{306} +3040^\circ$ ,  $[\phi]_{247} +9560^\circ$ . O.R.D. in isoctane (c 0.214, 420–240  $\mu$ m):  $[\phi]_{309} +2780^\circ$ ,  $[\phi]_{303} +2510^\circ$ ,  $[\phi]_{245} -7300^\circ$ . Ultraviolet spectrum in methanol:  $\lambda_{370-355}^{shoulder} \log \in 1.09-1.46$ ,  $\lambda_{279}^{max} \log \in 2.21$ . Ultraviolet spectrum in dioxane:  $\lambda_{340-310}^{shoulder} \log \in 1.00-1.52$ ,  $\lambda_{280}^{max} \log \in 2.10$ . Ultraviolet spectrum in isoctane:  $\lambda_{355-335}^{shoulder} \log \in 0.95-1.10$ ,  $\lambda_{280}^{max} \log \in 1.99$ .

**17 $\beta$ -Nitro-3 $\beta$ -acetoxy- $\Delta^5$ -androstene (XII)**<sup>25</sup> (Fig. 12): C.D. in methanol (c 1.042, 380–238  $\mu$ m):  $[\theta]_{380} 0$ ,  $[\theta]_{281} +8760$ ,  $[\theta]_{235} 0$ . O.R.D. in methanol (c 0.1042, 410–270  $\mu$ m); c 0.0208, 270–240  $\mu$ m):  $[\phi]_{345-325} +1250^\circ$ ,  $[\phi]_{304} +1940^\circ$ ,  $[\phi]_{250} -9730^\circ$ . Ultraviolet spectrum in methanol:  $\lambda_{360-350}^{shoulder} \log \in 1.26-1.44$ ,  $\lambda_{279}^{max} \log \in 2.07$ .

**17 $\beta$ -Nitro- $\Delta^4$ -androstene-3-one (XIII)**<sup>25</sup> (Fig. 13): C.D. in methanol (c 1.410, 385–310  $\mu$ m); c 0.282, 310–270  $\mu$ m); c 0.0564, 270–260  $\mu$ m):  $[\theta]_{385} 0$ ,  $[\theta]_{356} +160$ ,  $[\theta]_{320} -1420$ ,  $[\theta]_{278} +7230$ ,  $[\theta]_{266} +6100$ . O.R.D. in methanol (c 0.141, 410–340  $\mu$ m); c 0.047, 340–315  $\mu$ m); c 0.0156, 315–290  $\mu$ m); c 0.0031, 290–250  $\mu$ m):  $[\phi]_{362} +1305^\circ$ ,  $[\phi]_{349} +1125^\circ$ ,  $[\phi]_{300} +8010^\circ$ ,  $[\phi]_{257} -4050^\circ$ . Ultraviolet spectrum in methanol:  $\lambda_{315-275}^{shoulder} \log \in 2.09-2.70$ ,  $\lambda_{279}^{max} \log \in 4.15$ .

**6 $\beta$ -Nitro- $\Delta^4$ -androstene-3 $\beta$ ,17 $\beta$ -diol (XIV)**<sup>26</sup> (Fig. 14): C.D. in methanol (c 0.568, 390–300  $\mu$ m); c 0.142, 300–265  $\mu$ m); c 0.071, 265–240  $\mu$ m):  $[\theta]_{380} 0$ ,  $[\theta]_{288} -16050$ ,  $[\theta]_{256} 0$ ,  $[\theta]_{240} +5900$ . O.R.D. in methanol (c 0.225, 410–280  $\mu$ m); c 0.045, 280–230  $\mu$ m):  $[\phi]_{307} -10400^\circ$ ,  $[\phi]_{245} +22700^\circ$ . Ultraviolet in methanol:  $\lambda_{300-270}^{shoulder} \log \in 2.11-2.30$ .

**5 $\alpha$ -Chloro-6 $\beta$ -nitrocholestan-3 $\beta$ -ol acetate (XV)**<sup>27</sup> (Fig. 15): C.D. in methanol (c 1.114, 340–250  $\mu$ m); c 0.223, 250–240  $\mu$ m):

(36) A. C. Ott, M. F. Murray, and R. L. Pederson, *J. Am. Chem. Soc.*, **74**, 1239 (1952).



$[\theta]_{340}^0$ ,  $[\theta]_{287}^0 - 1100$ ,  $[\theta]_{240}^0$ . O.R.D. in methanol ( $c$  0.948, 410–280  $m\mu$ ;  $c$  0.0474, 280–240  $m\mu$ ):  $[\phi]_{311}^0 - 7950^\circ$ ,  $[\phi]_{305}^0 - 8030^\circ$ ,  $[\phi]_{280}^0 + 7500^\circ$ . Ultraviolet spectrum in methanol:  $\lambda_{272}^{max} \epsilon$  97.

**6 $\beta$ -Nitrotestosterone acetate (XVI)<sup>26</sup> (Fig. 16):** C.D. in methanol ( $c$  0.775, 400–330  $m\mu$ ;  $c$  0.086, 330–275  $m\mu$ ;  $c$  0.0215, 275–260  $m\mu$ ;  $c$  0.01075, 260–240  $m\mu$ ):  $[\theta]_{400}^0$ ,  $[\theta]_{375}^0 + 240$ ,  $[\theta]_{357}^0$ ,  $[\theta]_{281}^0 - 16200$ ,  $[\theta]_{260}^0$ ,  $[\theta]_{240}^0 + 15100$ . O.R.D. in methanol ( $c$  0.235, 400–290  $m\mu$ ;  $c$  0.0147, 290–230  $m\mu$ ):  $[\phi]_{302}^0 - 6560^\circ$ . Ultraviolet spectrum in methanol:  $\lambda_{232}^{max} \log \epsilon$  4.02.

**6 $\beta$ -Nitro-3 $\alpha$ ,5 $\alpha$ -cycloandrostan-17-one (XVII)<sup>29</sup> (Fig. 17):** C.D. in methanol ( $c$  0.708, 350–240  $m\mu$ ):  $[\theta]_{350}^0$ ,  $[\theta]_{326}^0 + 540$ ,  $[\theta]_{321}^0$ ,  $[\theta]_{305}^0 + 3120$ ,  $[\theta]_{288}^0$ ,  $[\theta]_{272}^0 - 2160$ ,  $[\theta]_{245}^0$ ,  $[\theta]_{240}^0 + 780$ . O.R.D. in methanol ( $c$  0.0804, 350–235  $m\mu$ ):  $[\phi]_{316}^0 + 1900^\circ$ ,  $[\phi]_{287}^0 - 3240^\circ$ ,  $[\phi]_{247}^0 + 1080^\circ$ . Ultraviolet spectrum in methanol:  $\lambda_{288}^{max} \log \epsilon$  2.11.

**6-Nitro- $\Delta^5$ -androstene-3 $\beta$ ,17 $\beta$ -diol (XVIII)<sup>26</sup> (Fig. 18):** C.D. in methanol ( $c$  0.860, 420–365  $m\mu$ ;  $c$  0.172, 365–300  $m\mu$ ;  $c$  0.0344, 300–240  $m\mu$ ):  $[\theta]_{410}^0$ ,  $[\theta]_{385}^0 + 170$ ,  $[\theta]_{375}^0$ ,  $[\theta]_{317}^0 - 2170$ ,  $[\theta]_{290}^0$ ,  $[\theta]_{265}^0 + 6640$ . O.R.D. in methanol ( $c$  0.068, 420–320  $m\mu$ ;  $c$  0.0136, 320–310  $m\mu$ ):  $[\phi]_{355}^0 - 2950^\circ$ ,  $[\phi]_{315}^0$ . Ultraviolet spectrum in methanol:  $\lambda_{370-350}^{absorber} \log \epsilon$  2.00–2.51,  $\lambda_{258}^{max} \log \epsilon$  3.54.

**6-Nitro- $\Delta^5$ -pregnen-3 $\beta$ -ol-20-one acetate (XIX)<sup>26</sup> (Fig. 19):** C.D. in methanol ( $c$  0.3626, 400–310  $m\mu$ ;  $c$  0.0725, 310–260  $m\mu$ ;  $c$  0.03625, 260–240  $m\mu$ ):  $[\theta]_{395}^0$ ,  $[\theta]_{328}^0 - 3180$ ,  $[\theta]_{313}^0$ ,  $[\theta]_{282}^0 + 1180$ ,  $[\theta]_{243}^0$ . O.R.D. in methanol ( $c$  0.0710, 410–340  $m\mu$ ;  $c$  0.0180, 340–290  $m\mu$ ;  $c$  0.0090, 290–270  $m\mu$ ;  $c$  0.00225, 270–230  $m\mu$ ):  $[\phi]_{384}^0 - 27400^\circ$ ,  $[\phi]_{304}^0 + 20300^\circ$ ,  $[\phi]_{230}^0 - 23600^\circ$ . Ultraviolet spectrum in methanol:  $\lambda_{350-326}^{absorber} \log \epsilon$  2.41–2.53,  $\lambda_{258}^{max} \log \epsilon$  3.28.

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## The Mechanism of Hydrolysis of Schiff Bases Derived from Aliphatic Amines<sup>1</sup>

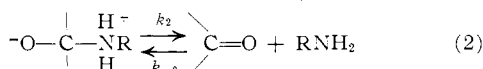
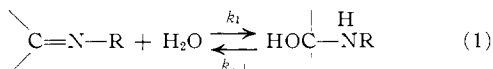
BY E. H. CORDES AND W. P. JENCKS

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The rates of hydrolysis of a series of *p*- and *m*-substituted benzylidene-1,1-dimethylethylamines have been investigated in the pH range 0–14 at 25°. The rates of hydrolysis of these Schiff bases are independent of pH above pH 9 and increase slightly with electron-donating power of the polar substituent. These results suggest that, under these conditions, the attack of hydroxide ion on the protonated Schiff base is the rate-determining step. Under more acidic conditions, in which an appreciable fraction of the Schiff base exists as the conjugate acid, the hydrolysis rates increase with decreasing pH for Schiff bases possessing an electron-withdrawing substituent and decrease with decreasing pH for Schiff bases possessing an electron-donating substituent. The changes in rate with pH are correlated with the conversion of the Schiff bases to their conjugate acids, indicating that the predominant reaction under these conditions is the attack of water on the protonated Schiff base. Below pH 4, the hydrolysis rates for all of the Schiff bases decrease and eventually become linear in respect to hydroxide ion concentration. This behavior is interpreted as a transition in rate-determining step from attack of water under neutral and basic conditions to decomposition of the tetrahedral addition intermediate under acidic conditions.

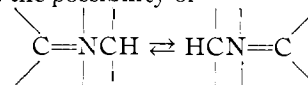
### Introduction

The formation and hydrolysis of a number of Schiff bases, oximes, semicarbazones, and related compounds has been shown to proceed through eq. 1 and 2, with step 1 rate-determining at neutral and alkaline pH



values and step 2 rate-determining at acidic pH values.<sup>2–8</sup> Although the formation and hydrolysis of benzylideneanilines has been studied in some detail, much less information is available regarding the reactions of Schiff bases formed from more basic amines. Morton and Pitt have shown that the rate of retinylidene-methylamine hydrolysis is much slower in acid than in neutral solution<sup>9</sup> and this behavior has been interpreted in terms of a change in rate-determining step from 1 to 2 at low pH values.<sup>5</sup> The condensation of ammonia and primary amines with aromatic aldehydes in methanol proceeds at a rate proportional to the concentrations of the un-ionized reactants and is subject to general acid catalysis by acetic acid.<sup>10–12</sup> The experiments reported here were carried out to ob-

tain further information on the mechanism and catalysis of the hydrolysis of Schiff bases formed from strongly basic amines. Such Schiff bases are of biological importance in a large number of enzymic reactions involving pyridoxal phosphate,<sup>13,14</sup> enzymic aldolization,<sup>15–17</sup> and decarboxylation<sup>18,19</sup> reactions and, probably, in the visual pigment, rhodopsin.<sup>20–21</sup> The experiments were carried out with Schiff bases formed from substituted benzaldehydes and *t*-butylamine in order to avoid the possibility of



tautomerization; subsequent studies with the corresponding ethylamine Schiff bases showed that this precaution was probably unnecessary, because the hydrolysis of these compounds proceeds in a manner similar to that of the *t*-butylamine compounds, without detectable tautomerization.

### Experimental

Kinetic measurements were carried out spectrophotometrically with a Zeiss PMQ II spectrophotometer equipped with a cell holder maintained at 25° as previously described.<sup>2–5</sup> In all

(1) Supported by grants from the National Science Foundation (GB-431 and G-14723) and the National Cancer Institute of the National Institutes of Health (CA-03975).

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