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The Ultraviolet Absorption Spectra of Steroidal Dinitrophenylhydrazones

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2,4-Dinitrophenylhydrazones are employed widely as carbonyl derivatives in view of their comparatively high melting points and easy crystallizability; furthermore, they are particularly useful in the chromatographic separation of a variety[1,2] of ketones including those of the steroid series.[3,4] Correlations between the ultraviolet absorption spectra of dinitrophenylhydrazones and the structure of the carbonyl compounds from which they are derived are therefore of obvious significance. Important contributions on this subject have been published recently by Braude and Jones,[5] and Roberts and Green,[6] who investigated the spectra of a considerable number of dinitrophenylhydrazones of representative saturated and unsaturated carbonyl compounds in both the aliphatic and aromatic series.

In connection with a study of the reaction of dinitrophenylhydrazine with steroidal bromo ketones,[7] it was necessary for comparison purposes to prepare authentic samples of a number of dinitrophenylhydrazones of steroid ketones with varying degrees of unsaturation and to determine their ultraviolet absorption curves. The present series of compounds is structurally more closely related than the hydrazones studied previously[5,6] and it was thus possible to examine the influence of relatively minor changes in the ketone moiety upon the absorption of the dinitrophenylhydrazone. Furthermore, precise absorption data for steroidal dinitrophenylhydrazones are lacking and in conjunction with the spectra of the carbonyl compounds should prove useful in the characterization of unknown steroid ketones or in the chromatographic separation of ketone mixtures *via* their dinitrophenylhydrazones.

Experimental

In the present investigation all determinations were carried out in chloroform because of solubility considerations, although ethanol, employed by Roberts and Green,[6] has the considerable advantage that the effect of alkali on the spectrum can also be determined. The spectrum of each com-

TABLE I

ABSORPTION SPECTRA OF STEROIDAL DINITROPHENYLHYDRAZONES

Dinitrophenylhydrazones of 3-ketosteroids	C-17 Substituents	λ max.,[a] mμ	λ max. of ketone,[b] mμ
Saturated, *allo*	C_8H_{17}, $COOCH_3$, $OCOC_6H_{11}$	367–369[c]	280–286
Δ^1-Unsaturated, *allo*	C_8H_{17}, $COOCH_3$, OCO_6H_{11}	382–384	230–232
2-Bromo-Δ^1-unsaturated, *allo*	C_8H_{17}, OCO_6H_{11}	375–376	254–256
Δ^4-Unsaturated	C_8H_{17}, (12-hydroxy)-cholenate	390[d]	240–242
$\Delta^{1,4}$-Unsaturated	C_8H_{17}, $COOCH_3$, OCO_6H_{11}	400–402	243–244
$\Delta^{4,6}$-Unsaturated	C_8H_{17}, $COOCH_3$, $OCOC_6H_5$	402–404	282–284

[a] The data refer to chloroform solutions and the typical log E values can be obtained from Figs. 1 and 2. [b] In ethanol solution. [c] Sitostanone dinitrophenylhydrazone is reported (ref. 5) with max. at 368 mμ. [d] As in all other cases, all values refer only to data obtained by us on our compounds on a standardized Beckman spectrophotometer. Jones, *et al.*, ref. 4, give a max. at 391 mμ for Δ^4-cholestenone and 392 mμ for the hydrazones of three other Δ^4-3-ketosteroids. For the same ketones Braude and Jones, ref. 5, report maxima ranging from 393–395 mμ. We consider the values of Jones, *et al.*, more reliable.

TABLE II

ABSORPTION SPECTRA OF MISCELLANOUS HYDRAZONE DERIVATIVES

No.	Compound[a]	λ max., mμ	Log E_m (chloroform)
1	Δ^5-Pregnen-3-ol-20-one DNPH	368	4.46
2	Progesterone bis-DNPH[b]	383	4.72
3	4,4-Dimethyl-1-keto-1,2,3,4-tetrahydronaphthalene DNPH[c]	385	4.49
4	4,4-Dimethyl-1-keto-1,4-dihydronaphthalene DNPH[c]	404	4.55
5	α-Ionone DNPH	387	4.49
6	β-Ionone DNPH	392	4.46
7	Benzalacetone DNPH	305 396[d]	4.16 4.53
8	$\Delta^{1,1'}$-2'-Keto-3,4-dihydro-1,2-cyclopentenonaphthalene DNPH[e]	312 404	4.17 4.70
9	3-(*p*-Methoxyphenyl)-cyclopenten-2-one-1 DNPH[e]	315 414	4.19 4.64
10	Cholestanone α-Methyl-DNPH	387.5	4.25
11	Δ^1-Cholestenone α-Methyl-DNPH	392.5	4.30
12	Δ^1-2-Bromocholestenone α-Methyl-DNPH	392	4.22

[a] DNPH denotes dinitrophenylhydrazone. [b] The spectrum of this compound has already been discussed by Djerassi, *Anal. Chem.*, **20**, 880 (1948). [c] Both dinitrophenylhydrazones were kindly supplied by J. S. Buckley, Jr., of the University of Minnesota [Arnold, Buckley and Richter, THIS JOURNAL, **69**, 2322 (1947)]. [d] In ethanol solution the maxima were shifted to 302 mμ (log E 4.23) and 393 mμ (log E 4.57), respectively. [e] Compounds 8 and 9 were furnished by Prof. A. L. Wilds of the University of Wisconsin [Wilds, T. L. Johnson and J. A. Johnson, THIS JOURNAL, **67**, 286 (1945); **68**, 86 (1946)].

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[1] See Roberts and Green, *Ind. Eng. Chem., Anal. Ed.*, **18**, 335 (1946), and White, *Anal. Chem.*, **20**, 726 (1948), for leading references.

[2] Gaddis and Butz, THIS JOURNAL, **69**, 117 (1947); Butz, Davis and Gaddis, *J. Org. Chem.*, **12**, 122 (1947).

[3] Johnston, *Science*, **106**, 91 (1947).

[4] Jones, Wilkinson and Kerlogue, *J. Chem. Soc.*, 391 (1942).

[5] Braude and Jones, *ibid.*, 498 (1945).

[6] Roberts and Green, THIS JOURNAL, **68**, 214 (1946).

[7] Djerassi, *ibid.*, **71**, 1003 (1949).

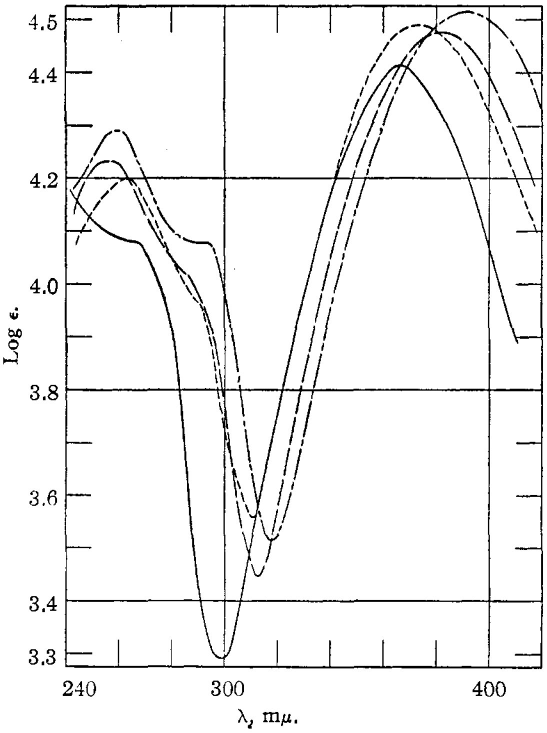

Fig. 1.—Ultraviolet absorption spectra in chloroform solution of dinitrophenylhydrazones of: — saturated 3-ketosteroids; — — — Δ^1-3-ketosteroids; - - - - - - Δ^1-2-bromo-3-ketosteroids; — — — — — Δ^4-3-ketosteroids.

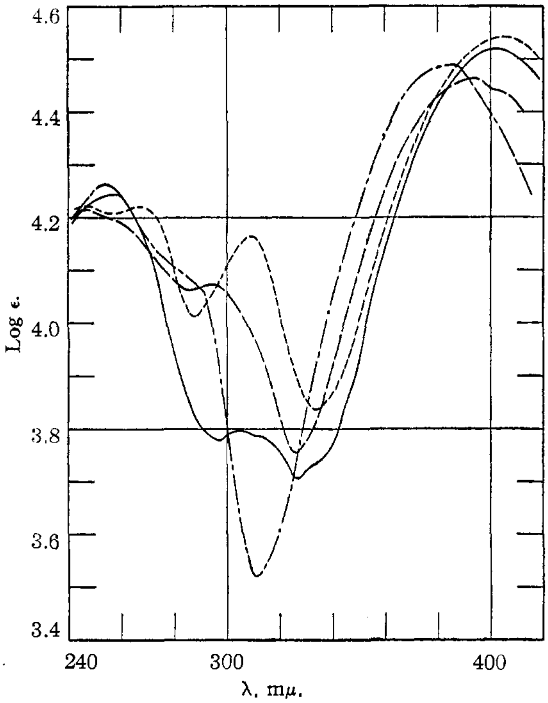

Fig. 2.—Ultraviolet absorption spectra in chloroform solution of dinitrophenylhydrazones of: — $\Delta^{1,4}$-3-keto-steroids; - - - - - - - $\Delta^{4,6}$-3-ketosteroids; — — — — — — — α-ionone; — — — β-ionone.

pound was examined in a Beckman quartz spectrophotometer in the region 252 mμ to 415 mμ at 2.5 mμ intervals, except around maxima and minima where 1 mμ intervals were used; $E_m = 1/c \log I_0/I$, where c is the concentration in moles per liter. All samples were of analytical purity and in most instances were prepared by two different methods as described elsewhere.[7]

Discussion

It is apparent from an inspection of Table I and Figs. 1 and 2 that the main maxima of the dinitrophenylhydrazones of the various types of ketones in the steroid series do not overlap and differ sufficiently so that they may be correlated with the structure of the ketone from which the hydrazone is prepared. The various factors contributing to the absorption spectra of dinitrophenylhydrazones have already been discussed by earlier workers,[5,6] but it will be necessary to revise, or at least amplify somewhat, the position of the maxima as given by them for the various types of carbonyl derivatives. In the case of the wave length limits given by Roberts and Green,[6] a slight expansion is necessary to accommodate certain unsaturated ketones and to apply the data to chloroform solutions. It is suggested that this solvent should be used in preference to ethanol, since many dinitrophenylhydrazones are insoluble in the latter and no definite correction can be made for shifts in the two solvents.[8] The values of Braude and Jones[5] for compounds which we have checked, occasionally needed corrections ranging from 2–5 mμ,[9] either reflecting differences in the purity of the samples or in the spectrophotometers. These experimental differences as well as the fact that our narrow wave length limits given in Table I refer only to steroid ketones (cyclic six-membered), while those of the English workers include predominantly open-chain derivatives, account for the differences in our respective wave length ranges for unsaturated ketones.

While saturated 3-ketosteroids possess a low

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[8] It should be noted that although no correction appears necessary in changing from ethanol to chloroform in the case of unsaturated ketones [Woodward, THIS JOURNAL, 63, 1123 (1941)], erratic shifts are obtained in the case of dinitrophenylhydrazones as shown below (*cf.* ref. 5), thus making it desirable to employ only one solvent. The unpublished results of Roberts and Trimble were kindly supplied by Dr. John D. Roberts of M. I. T.

	λmax ($EtOH$) (ref. 5)	λmax ($CHCl_3$) (ref. 5)	λmax ($EtOH$) (ref. 6)	λmax ($CHCl_3$) (Roberts and Trimble)
2,4-Dinitrophenylhydrazine	350	343.5	352	341
Formaldehyde DNPH	348	348	349	345
Acetaldehyde DNPH	360	360	356	356
Crotonaldehyde DNPH	373	372	377	374
Chalcone DNPH	...	...	395	398

We have confirmed Roberts' results for dinitrophenylhydrazine and the shift in the case of chalcone DNPH is quite similar to that observed in the case of benzalacetone DNPH (Table II).

[9] In that connection, see Jones, *et al.*, ref. 4, Barton and Jones, *J. Chem. Soc.*, 599 (1943), and the comparison with Roberts' results (footnote 8, this paper).

maximum at *ca.* 280–286 mμ (log *E ca.* 1.5),[10] the corresponding dinitrophenylhydrazones show a pronounced maximum (log *E* 4.4) at 367–369 mμ,[11] thus constituting a good diagnostic test for this type of carbonyl compound The introduction of one double bond results in a bathochromic shift of about 15–20 mμ, thus again permitting ready differentiation from the corresponding saturated derivatives. The dinitrophenylhydrazones of such singly unsaturated ketones can be separated into two classes on the basis of the absorption spectra although the difference (6–8 mμ) between the maxima of the hydrazones of the Δ^1-ketones (one β-substituent and no exocyclic double bond) and the Δ^4-ketones (two β-substituents and one exocyclic bond) is not as pronounced as in the corresponding ketones (10–15 mμ).[12] This is understandable since it has been noted already[5,6] that relatively minor changes in the carbonyl part of the molecule are not too readily transmitted over the =N–NH–bond. Of considerable interest is the fact that the introduction of an additional α,β-conjugated double bond next to the keto group exerts a pronounced bathochromic shift. Thus while Δ^4-3-ketosteroids and the corresponding $\Delta^{1,4}$-dien-3-ones show practically the same selective absorption (*ca.* 242 mμ), the maxima of their dinitrophenylhydrazones differ markedly (390 mμ as compared to 404 mμ) and this should prove quite useful for purposes of differentiation between those two types of compounds (see Table I). It is also interesting to note that in the case of the hydrazones, both the cross-conjugated $\Delta^{1,4}$-dienone and the linear conjugated $\Delta^{4,6}$-dienone derivatives possess nearly the same maximum (around 404 mμ), whereas the spectra of the parent dienones differ very considerably (by about 40 mμ, see Table I). The hydrazones of those two types of dienones can be differentiated, however, by their spectra in the region 280–330 mμ,[13] since the dinitrophenylhydrazones of the $\Delta^{4,6}$-dienones exhibit a new and characteristic maximum at 309 mμ (Fig. 2).

It is of interest to note that the maximum of the Δ^1-2-bromo-3-keto*allo*steroid dinitrophenylhydrazones occurs at an appreciably lower wave length (*cf.* Table I) than that of the Δ^1- and Δ^4-unsaturated hydrazones,[14] which is in contrast to the absorption spectra of the corresponding ketones, where the Δ^1-2-bromo-3-ketones absorb at a higher wave length.[15]

The spectra of some miscellaneous dinitrophenylhydrazones are recorded in Table II. Through the kind coöperation of Drs. H. Sobotka and J. D. Chanley of the Mt. Sinai Hospital, New York City, we obtained authentic samples[16] of the dinitrophenylhydrazones of α- and β-ionone (compounds 5 and 6, Table II). Their spectra are reproduced in Fig. 2 and corroborate the previous report[5] that β-ionone dinitrophenylhydrazone lacks a definite maximum at 309 mμ in contrast to the hydrazones of ψ-ionone or the $\Delta^{4,6}$-3-ketosteroids discussed above, even though all three possess the same conjugated system. We did observe, however, that the β-isomer showed a slightly higher absorption maximum (392 mμ) than the α-isomer (387 mμ).[16a] It is probably not necessary to assume that some isomerization of the β-isomer had occurred during the preparation of its dinitrophenylhydrazone in glacial acetic acid, in order to explain the lowered maximum (392 as compared to *ca.* 404 mμ) and the absence of the maximum at 309 mμ, since β-ionone appears to be stable in acid solution.[17] It is simply a further instance of the anomalous spectral behavior of β-ionone, as exemplified by the ionone semicarbazones[17,18] and ionylideneacetones,[19] which has been ascribed[18] in part to a steric effect of the methyl group.

Qualitatively, a benzene ring adjacent to a carbonyl group can be considered equivalent (as a chromophore) to a double bond[20] and on that basis the two naphthalene derivatives 3 and 4 (Table II) represent a single unsaturated ketone and a cross-conjugated dienone, respectively. The spectra of their dinitrophenylhydrazones differ in the same manner as the comparable steroid derivatives discussed above.

The light absorption of the dinitrophenylhydra-

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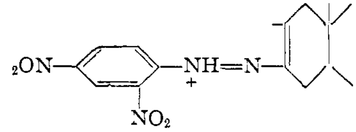
[10] *E. g.*, cholestanone, max. at 286 mμ (log *E ca.* 1.3) [Klotz, THIS JOURNAL, **66**, 90 (1944)] and dihydrotestosterone hexahydrobenzoate, max. at 280.5 mμ (log *E* 1.49) [Wilds and Djerassi, *ibid.*, **68**, 2128 (1946)].

[11] The maxima for the dinitrophenylhydrazones of cyclohexanone (366 mμ) and sitostanone (368 mμ) (ref. 5) are in agreement with this classification.

[12] *Cf.* Woodward, THIS JOURNAL, **63**, 1123 (1941); **64**, 76 (1942); and Gillam, *et al.*, *J. Chem. Soc.*, 815 (1941); 486 (1942), for correlation of absorption spectra and structure of ketones.

[13] This region cannot be employed for the characterization of the other hydrazones, since their spectra coincide almost completely with that of dinitrophenylhydrazine. This region of the spectrum of dinitrophenylhydrazine is discussed in detail by Braude and Jones (ref. 5).

[14] The influence of substituents in the carbonyl moiety of dinitrophenylhydrazones on the relative importance of contributing forms to the resonance hybrid has been discussed by Roberts and Green (ref. 6). It seems likely that the effect of the bromine atom is to favor a form such as



at the expense of quinoid structures with longer conjugated systems.

[15] It has been pointed out [Djerassi and Scholz, THIS JOURNAL, **69**, 2405 footnote 5 (1947)] that the Δ^1-2-bromo-3-ketones do not follow Woodward's rules (ref. 12) with respect to their absorption maxima.

[16] Sobotka, Bloch and Glick, THIS JOURNAL, **65**, 1961 (1943).

[16a] NOTE ADDED IN PROOF: Since the submission of this manuscript, a paper by Naves and Ardizio [*Helv. Chim. Acta*, **31**, 1926 (1948)] appeared in which the ultraviolet absorption spectra of the dinitrophenylhydrazones of α-ionone (max. at 376 mμ) and β-ionone (max. at 385 mμ) were determined in ethanol solution. This serves as a furthes illustration of the irregular shifts observed in changing from ethanol to chloroform (*cf.* footnote 8).

[17] *Cf.* Young, Cristol, Andrews and Lindenbaum, *ibid.*, **66**, 855 (1944).

[18] Burawoy, *J. Chem. Soc.*, 20 (1941).

[19] Young and Linden, THIS JOURNAL, **69**, 2042 (1947) and references cited therein.

[20] See for instance Wilds, Beck, Close, Djerassi, Johnston, Johnson and Shunk, *ibid.*, **69**, 1991 (1947).

zones of benzalacetone (no. 7) and of the cyclopentenonaphthalene derivative 8 resembles that of linear conjugated dienones by exhibiting the characteristic maximum between 305–312 mμ in addition to the one at 396–404 mμ. The introduction of a *p*-methoxy group into such a system (no. 9) results in an expected bathochromic shift.[21] A considerable number of compounds would be required, however, to establish definite figures on the effect of a five-membered ring or ring substituents on the absorption of such aromatic dinitrophenylhydrazones.

In Table II are also listed the spectra of three α-(2,4-dinitrophenyl)-α-methylhydrazones of the steroid series. Although a slight bathochromic shift with respect to the corresponding dinitrophenylhydrazones was to be expected by analogy to the behavior of the methylsemicarbazones,[22] the pronounced shift (10–20 mμ) which was observed was not anticipated. These derivatives crystallize only poorly in the steroid series and thus do not lend themselves readily to the characterization of unknown carbonyl derivatives.

Summary

As an additional tool for the characterization of steroid ketones, the ultraviolet absorption spectra of a number of representative saturated and unsaturated steroid 2,4-dinitrophenylhydrazones have been determined. The position of their maxima is discussed on the basis of earlier work on the light absorption of such carbonyl derivatives.

The spectra of certain other dinitrophenylhydrazones are also recorded, including those of α- and β-ionone.

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[21] A shift of the same magnitude was observed in the hydroxybenzaldehyde series (ref. 6).

[22] Evans and Gillam, *J. Chem. Soc.*, 565 (1943).

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The Scope and Mechanism of the Reaction of Dinitrophenylhydrazine with Steroidal Bromo Ketones[1]

BY CARL DJERASSI

Most of the physiologically potent steroids in the androgenic, progestational and cortical hormone series possess a Δ^4-3-keto grouping (IIa). Unless prepared from a steroid already possessing unsaturation in the 4-5 or 5-6 positions, the double bond is usually introduced by brominating the saturated 3-ketosteroid and dehydrobrominating the resulting bromo ketone, a method which is particularly important in the synthesis of the cortical hormones. Since 3-keto*allo*steroids (A:B ring juncture as in *trans* decalin) on monobromination give the 2-bromo derivative III,[2] in general only the ketones of the "normal" series (A:B juncture *cis*) have been employed for this purpose since they lead to 4-bromo-3- ketones I.[2,3] The dehydrobromination of the latter is usually accomplished by refluxing with pyridine for several hours[3,4] and it is believed[4] that frequently one of the factors responsible for the relatively poor yield is the formation of two diastereoisomeric 4-bromo derivatives, only one of which can undergo facile *trans*-elimination of hydrogen bromide. Recently, the mono- and dibromination products of the *allo* series have gained increased importance, since the 2,4-dibromo-3-ketosteroids (VII) on dehydrobromination yield the 1,4-dienones VIIIa, which represent the key intermediates in the partial synthesis of the estrogens.[5,6] Furthermore, partial dehydrobromination of the dibromo ketones (VII) leads to the 2-bromo-Δ^4-3-ketosteroids (IX)[7,8] which can be converted to Δ^4-3-ketosteroids (IIa). Even the 2-bromo-3-keto*allo*steroids (III) can be transformed partially to the Δ^4-ketones[8,9] although the main product is the Δ^1-isomer (IVa). Although Butenandt and co-workers[10] have shown that collidine is much more effective in the dehydrobromination of brominated derivatives of the *allo* series than is pyridine, the latter is still the base of choice for normal ketones. In spite of the importance of dehydrobrominations in steroid chemistry, even in the most thoroughly studied cases (both *allo* and normal series) using either collidine or pyridine, the yield in that step has never surpassed 60% which in turn has been a serious drawback in a number of syntheses.

A few months ago, Mattox and Kendall[11] in a preliminary communication recorded the interesting observation that when certain cortical hormone intermediates containing the 3-keto-4-bromo grouping (I) were treated in acetic acid solution with 1.2 moles of dinitrophenylhydrazine

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[1] Presented in part on the program of the Division of Medicinal Chemistry at the Washington, D. C., meeting of the American Chemical Society, August 31, 1948.

[2] Butenandt and Wolff, *Ber.*, **68**, 2091 (1935).

[3] Butenandt and Schmidt, *ibid.*, **67**, 1901 (1934).

[4] v. Euw and Reichstein, *Helv. Chim. Acta*, **29**, 654 (1946).

[5] Inhoffen, *Angew. Chem.*, **59A**, 207 (1947).

[6] Wilds and Djerassi, THIS JOURNAL, **68**, 2125 (1946).

[7] Inhoffen and Zuehlsdorff, *Ber.*, **76**, 233 (1943).

[8] Djerassi and Scholz, THIS JOURNAL, **69**, 2404 (1947); *J. Org. Chem.*, **13**, 697 (1948).

[9] Ruzicka, Plattner and Aeschbacher, *Helv. Chim. Acta*, **21**, 866 (1938); Marker, Wittle and Plambeck, THIS JOURNAL, **61**, 1333 (1939).

[10] Butenandt, Mamoli, Dannenberg, Masch and Paland, *Ber.* **72**, 1617 (1939).

[11] Mattox and Kendall, THIS JOURNAL, **70**, 882 (1948).