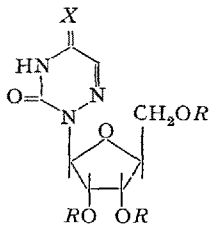


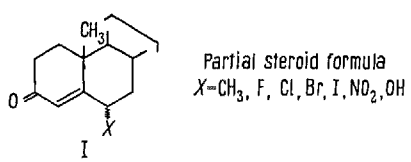
I (X = O; R = Benzoyl)
II (X = S; R = Benzoyl)
III (X = NH; R = H)
IV (X = S; R = H)

Zusammenfassung. 6-Azacytidin wurde synthetisiert; es erwies sich als Antimetabolit.

F. Šorm, J. Smrt, and V. Černeckij[3]
Department of Organic Synthesis, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science, Prague, November 3, 1960.

The Ultraviolet Light Absorption Spectra of Steroid C-6 Substituted-Δ^4-3-Ketones[1]

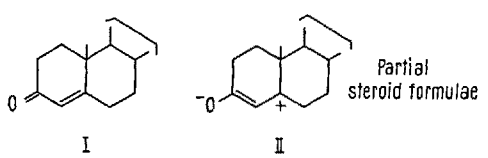
The basic structures under consideration are illustrated by formula I[2].



I

In the absence of extraneous factors (e.g. 11-keto substituents) a steroidal Δ^4-3-ketone, unsubstituted at C-2 or C-6, in alcohol solution exhibits maximum absorption in the ultraviolet at 240–242 mμ[3]. Substitution of electronegative groups at C-6 gives rise to either bathochromic or hypsochromic shifts in the absorption maximum. Before detailing the individual cases, the principal factors affecting the ultraviolet light absorption properties of C-6 substituted Δ^4-3-keto steroids will be discussed.

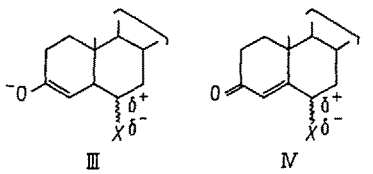
The excited state of Δ^4-3-keto steroids. The principal high intensity band of Δ^4-3-keto steroids observed in the 240 mμ region is the K band (N→V transition) and is attributed to the formation of the excited state (I→II) wherein the charged dipolar form dominates.



I II

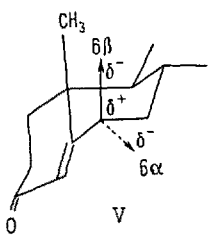
Possible factors affecting transition energy (ΔE) of C-6 substituted Δ^4-3-keto steroids. a) *Planarity.* For effective electronic transition between the ends of a dipole it is necessary that the chromophore, in this case the unexcited unsaturated ketone, be essentially uniplanar. Steric factors which result in distortion of the chromophore plane may affect the energy requirement necessary to reach the excited state. *A priori* it might have been anticipated that a bulky 6β-axial substituent would cause a distortion of the chromophore by virtue of the 1, 3-diaxial non-bonded interactions between such a group and the C-10 methyl group. Anticipating the discussion which follows it may be noted that the effect on the ultraviolet maximum of this 1, 3-diaxial interaction can only be minimal.

b) *Inductive effect.* The inductive effect of an electron-withdrawing group γ-substituted to an α,β-unsaturated ketone will oppose the polarization inherent in the excited state by charge repulsion between the C-5 and C-6 electron deficient centres (cf. III). Further, an electronegative γ substituent will, by placement of a partial positive charge on the γ-carbon atom (cf. IV) oppose the mobilization of the π-electrons necessary to reach the excited state.



III IV

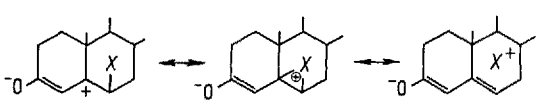
The geometry of the system is such that if the substituent at C-6 occupies the β-axial position its interaction with the dipole of the α,β-unsaturated ketone system, is of greater magnitude than that of an identical substituent in the α-equatorial position, cf. Figure V. Thus the inductive effect of a 6β substituent will be greater than that of the corresponding 6α substituent.



V

Although no such cases have been reported, one may predict that C-6 substitution of a Δ^4-3-ketone by a strong electron releasing group would result in a lowering of the excitation energy and a consequent bathochromic shift in the position of maximum absorption (K band) in the ultraviolet spectrum.

c) *Neighbouring group participation in the excited state.* If a substituent at C-6 is in such a position that it may with minimal movement interact with the unshared orbital of the excitation state C-5 carbonium ion and if the substituent is capable of accepting a positive charge, participation in the excited state is possible (Fig. VI).



VI

This will result in a lowering of the excitation energy due to an extension of the effective length of the conjugated

[1] This paper is considered to be Steroids CLXI in the series from these laboratories; part CLX, C. Casas Campillo and L. F. Bojalil, American Review of Respiratory Diseases, in press.
[2] For leading references to the preparation of compounds of this type cf. A. Bowers, E. Denot, M. B. Sanchez, L. M. Sanchez-Hidalgo, and H. J. Ringold, J. Amer. chem. Soc. *81*, 5233 (1959).
[3] For an excellent compendium and review of the ultraviolet absorption spectra of steroids, covering the literature into 1952, see L. Dorfman, Chem. Rev. *53*, 47 (1953).

system[4] (as well as resonance stabilization of the excited state).

It would appear that such participation is a major factor in the ultraviolet maximum of 6β-chloro, bromo and iodo-Δ^4-3-keto steroids.

6-Methyl-Δ^4-3-ketones. It is evident from the Table that a methyl substituent in either the 6α- or 6β-configuration exerts little or no effect on the unsaturated ketone absorption band in the 240 mμ region. This indicates that the weak electron releasing properties of this group are insufficient to exert any bathochromic spectral shift. No other factor need be considered in interpreting the spectral maximum of a 6-methyl-Δ^4-3-ketone.

6-Halo-Δ^4-3-ketones. Substitution by a 6α-halo group results in a hypsochromic shift of −4 to −5 mμ in the case of fluoro or chloro and a slightly lower shift of −3 to −4 mμ in the case of bromo. It is clear that this change is due to the inductive effect of the halogen substituents (formulae III and IV) increasing the excitation state energy requirement. The minimal difference in the effects of the three halogen substituents is paralleled by the essentially similar ionization constants of the corresponding α-halo acetic acids, acetic acid pK 4.76, α-fluoroacetic acid pK 2.66, α-chloroacetic acid 2.81, α-bromoacetic acid 2.87.

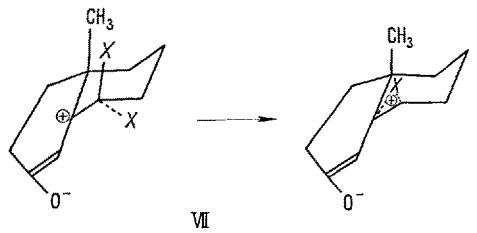
With respect to 6β-halo substituents it is apparent that in some cases more than one effect is operative. A hypsochromic effect of −5 to −8 mμ is noted for fluorine, essentially no effect is found for chlorine (0 to −1) while a bathochromic effect of +6 to +8 mμ for bromine and +11 to +13 mμ for iodine is noted. In the case of 6β-fluoro (axial) it appears that the inductive effect is the only important factor to be considered. The hypsochromic shift is greater than that of the α-fluoro substituent in agreement with the considerations noted above. By analogy with the fluoro case the inductive effect of the 6β-chloro, bromo and iodo atoms must be as great or greater than that of the corresponding 6α-substituent but this electron withdrawal effect is completely overshadowed by a second effect.

Influence of C-6 substituent on principal ultraviolet maximum of Δ^4-3-keto steroids

Substituent at C-6	$\Delta\lambda_{max}$ α	$\Delta\lambda_{max}$ β
CH_3[a]	0 to +1	−1 to +1
F[b]	−4 to −5	−5 to −8
Cl[c]	−4 to −5	0 to −1
Br[d]	−3 to −4	+6 to +8
I[d]	No data	+11 to +13
OH[e]	0 to −1	−3 to −6
NO_2[f]	−7 to −9	−6 to −8

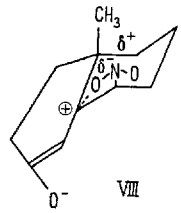
[a] H. J. RINGOLD, E. BATRES, and G. ROSENKRANZ, J. org. Chem. *22*, 99 (1957).
[b] A. BOWERS, L. C. IBÁÑEZ, and H. J. RINGOLD, Tetrahedron *7*, 138 (1959).
[c] H. J. RINGOLD, E. BATRES, A. BOWERS, J. EDWARDS, and J. ZDERIC, J. Amer. chem. Soc. *81*, 3485 (1959) and unpublished observations from these laboratories.
[d] A. BOWERS, E. DENOT, and R. BECERRA, J. Amer. chem. Soc. *82*, 4001 (1960) and unpublished observations from these laboratories.
[e] cf. [3], p. 72.
[f] A. BOWERS, M. B. SÁNCHEZ, and H. J. RINGOLD, J. Amer. chem. Soc. *81*, 5234 (1959). – A. BOWERS, L. C. IBÁÑEZ, and H. J. RINGOLD, J. Amer. chem. Soc. *81*, 3707 (1959).

The second factor operative in the 6β-halo series is the participation (see Fig. VI) of the halogen atom in the excited state which results in a bathochromic shift compared to the α-substituent. Participation appears to be negligible in the case of fluorine and then increases with decreasing electronegativity of the halogen atom (i.e. I > Br > Cl). As pointed out above, the neighbouring group must be in such proximity to the unshared orbital of the C-5 transition state carbon atom that bond overlapping can occur with a minimum of movement. This requirement is readily met by a 6β-halo substituent while considerable atomic movement through space would be required by a 6α-halo substituent for participation in a uniplanar arrangement (Fig. VII). The size of the halo atom parallels the ability of the atom to accept a positive charge and both of these factors facilitate participation of the halogen atom in the excited state. Increasing participation lowers the excitation energy and raises the ultraviolet absorption maximum of the unsaturated ketone. The bathochromic effect compared to the C-6 unsubstituted compound is at a maximum of about 12 mμ in the case of iodine. This result is consistent with the work of Winstein demonstrating that iodine is a better participating group in neighbouring group reactions than bromine or chlorine[5].



VII

6-Nitro-Δ^4-3-ketones. The powerful electron withdrawing nitro group exerts, as expected, a marked hypsochromic shift ranging from −6 to −9 mμ. However, contrary to expectation, the 6β-axial substituent is not a more potent inductive group than the 6α-nitro group and the ultraviolet shift for the 6β-group (−6 to −8 mμ) is actually slightly less than for the 6α-group (−7 to −9 mμ). A possible explanation for this discrepancy may lie in charge stabilization of the C-5 transition state carbonium ion by one of the electronegative oxygen atoms of the nitro group. The geometry of this interaction would again be more favored for the axial nitro substituent than for the equatorial one, cf. Figure VIII.



VIII

[4] cf. The bathochromic effect of the primary hydroxyl group in substituted sorbyl and cinnamyl alcohols, E. H. BRAUDE and E. S. WAIGHT, *Progress in Stereochemistry* (Ed. W. KLYNE, Butterworths Scientific Publications, London 1954), Vol. I, Chapter 4, p. 154.
[5] S. WINSTEIN, J. Amer. chem. Soc. *64*, 2791 (1942) and subsequent papers.
[6] Present address, The Worcester Foundation for Experimental Biology, Shrewsbury (Mass., U.S.A.).
[7] *Acknowledgment.* The authors wish to thank Dr. G. STORK for helpful discussions.

6-Hydroxy-Δ^4-3-ketones. A 6β-hydroxy substituent exerts a hypsochromic effect of -3 to -6 mμ while a 6α-hydroxy substituent exhibits essentially no shift (0 to -1 mμ). Only inductive effects appear to be operative since it is apparent from these results that the 6β-hydroxyl group cannot be entering into a significant degree of participation.

The difference between hydroxyl group behaviour and halogen (e.g. chlorine or bromine) with respect to neighbouring group participation in the excited state is readily explained on the basis of the smaller size of the oxygen atom as well as its greater reluctance to accept a positive charge.

Zusammenfassung. Es werden die Hauptabsorptionsbanden (N→V Übergang) für eine Serie von Δ^4-3-Steroidketonen mit C-6-Substituenten diskutiert. Dabei wird versucht, die Lage der Hauptbanden dieser Verbindungen zu erklären. Besonders wird der Einfluss des induktiven Effekts und der von benachbarten Gruppen auf den angeregten Zustand berücksichtigt.

H. J. RINGOLD[6] and A. BOWERS[7]

The Research Laboratories, Syntex S.A., Mexico, D.F., September 5, 1960.

Determination of Pentoses

Many methods have been suggested for assaying the pentoses and particularly for ribose[1-7]. The number of procedures is an obvious indication of the difficulties of such a determination.

The method now used, with marked advantage over the other, is that of DISCHE and BORENFREUND[8], based on a modification of the phloroglucinol reaction of TOLLENS[9].

Even the investigators and originators of this method themselves admit that it is less sensitive (about half) than the orcinol method suggested by BIAL[1], but it has the enormous advantage of greater specificity. In consideration of the latter, we decided to see if it was possible to increase the sensitivity of the DISCHE-BORENFREUND method.

Our aim was reached after many attempts, by suitably modifying the original method of DISCHE and BORENFREUND. The modified method is of advantage for its increased sensitivity and consists in treating the sugar solution with concentrated HCl which doubles the intensity of the colour developed by pentose and phloroglucinol with respect to that obtained in the experimental conditions suggested by DISCHE and BORENFREUND.

New method. Reagents and technique. The new method requires the following reagents: a) Hydrochloric acid (d = 1.19); b) Glacial acetic acid; c) 0.8% aqueous glucose solution; d) Alcoholic solution of phloroglucinol, prepared by dissolving 5 g of phloroglucinol in 100 ml of ethanol 95°.

At the moment of use, 2 ml of hydrochloric acid (d = 1.19), 110 ml of glacial acetic acid, 1 ml of 0.8% glucose solution and 5 ml of alcoholic phloroglucinol solution are mixed. The mixture forms the phloroglucinol reagent and may be stored in a refrigerator at 0°C.

After preparing the reagent, 0.5 ml of a ribose solution containing from 5–25 μg of ribose are placed in a test-tube; 0.5 ml of HCl (d = 1.19) are added and the mixture shaken. Then 4.5 ml of the phloroglucinol reagent are added and the tube immersed in a boiling water bath for exactly 5 min. At the end of this period, the tube is taken out of the water-bath and immediately cooled in ice, remaining in ice until the spectrophotometric reading, which must be performed at 552 and 510 mμ, using a blank where the pentose has been replaced by 0.5 ml of distilled water. The differences between the values ob-

Tab. I. Comparison between the optical density obtained from D-ribose with the original DISCHE-BORENFREUND method and that as here modified

D-ribose in 5.5 ml final volume	DISCHE-BORENFREUND method			Modified DISCHE-BORENFREUND method		
	Optical density		Difference	Optical density		Difference
	552 mμ	510 mμ		552 mμ	510 mμ	
5 μg	0.089	0.026	0.063	0.134	0.035	0.099
10 μg	0.164	0.044	0.120	0.285	0.074	0.211
15 μg	0.238	0.064	0.174	0.435	0.116	0.319
20 μg	0.320	0.080	0.240	0.585	0.165	0.420
25 μg	—	—	—	0.705	0.190	0.515

Tab. II. Optical density (O.D.) obtained in treating 10 μg of ribose alone and in the presence of 30 μg of fructose, galactose, glucosamine, mannose and glucuronic acid, respectively, according to the phloroglucinol reaction

Quantity of sugar in 5.5 ml final volume	DISCHE-BORENFREUND method		Difference	Modified DISCHE-BORENFREUND method		Difference
	O.D. at 552 mμ	O.D. at 510 mμ		O.D. at 552 mμ	O.D. at 510 mμ	
Ribose 10 μg	0.145	0.035	0.110	0.280	0.065	0.215
Ribose 10 μg + fructose 30 μg	0.150	0.044	0.116	0.325	0.102	0.223
Ribose 10 μg + galactose 30 μg	0.150	0.037	0.113	0.305	0.087	0.218
Ribose 10 μg + glucosamine 30 μg	0.144	0.034	0.110	0.282	0.067	0.215
Ribose 10 μg + mannose 30 μg	0.155	0.040	0.115	0.325	0.095	0.230
Ribose 10 μg + Gluc. acid 30 μg	0.180	0.042	0.138	0.340	0.089	0.251

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[2] G. EMBDEN and M. LEHNARTZ, Z. physiol. Chem. *201*, 149 (1931).
[3] Z. DISCHE and K. SCHWARZ, Mikrochim. Acta 2, 13 (1937).
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[7] A. BONSIGNORE, G. CONTE, and M. ORUNESU, G. Biochim. *1*, 383 (1951–52).
[8] Z. DISCHE and E. BORENFREUND, Bioch. biophys. Acta *23*, 639 (1957).
[9] H. J. WHEELER and B. TOLLENS, Liebigs Ann. Chem. *254*, 329 (1889).