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.

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

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.

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

SUMMIT, NEW JERSEY RECEIVED SEPTEMBER 18, 1948

[CONTRIBUTION FROM THE DIVISION OF CHEMISTRY, RESEARCH DEPARTMENT, CIBA PHARMACEUTICAL PRODUCTS, INC.]

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

(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).

with or without the addition of sodium acetate, the dinitrophenylhydrazone IIb of the corresponding Δ^4-3-ketosteroid was obtained in excellent yield. Furthermore, on cleavage with pyruvic acid in the presence of hydrogen bromide, the unsaturated ketone could be regenerated almost quantitatively. Aside from the statement that "the reactivity of the bromine at C_4 is greatly increased through formation of a hydrazone at C_3," no mechanism was advanced, and no exact experimental details as to time or temperature were given. The purpose of the present paper is to record the results of an investigation of the scope of this reaction, particularly as applied to 3-keto*allo*steroids, and to suggest a reaction mechanism.

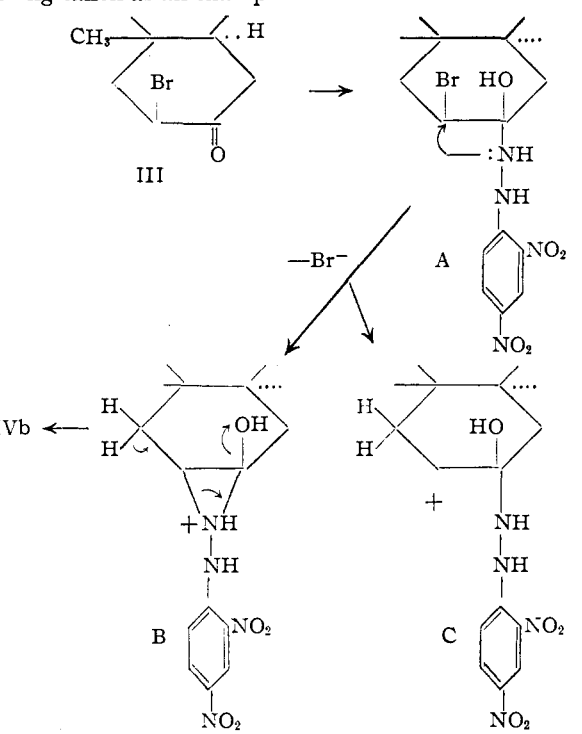
In the present investigation it was found that the reaction of the steroidal bromo ketone with 1.0–1.1 moles of dinitrophenylhydrazine was completed after heating for three to five minutes. Sodium acetate was not used so as not to introduce complications in a discussion of the reaction mechanism and those conditions were adhered to for all of the compounds described below.

Since 4-bromo-3-ketosteroids of the normal series (I) have already been studied by Mattox and Kendall,[11] only one additional example (methyl 3-keto-4-bromo-12-hydroxycholanate) was tested which gave nearly 90% of the dinitrophenylhydrazone IIb.

Three examples of 2-bromo-3-keto*allo*steroids (III) were investigated (R = C_8H_{17}, $COOCH_3$, $OCOC_6H_{11}$), all of which gave 80–90% of the dinitrophenylhydrazone IVb of the Δ^1-3-ketone. Just as with collidine,[7,8] 2,2-dibromoketones V (R = C_8H_{17}, $OCOC_6H_{11}$) led to the Δ^1-2-bromo derivatives VIb in 60% yield. In each case, the identity of the product was confirmed by analysis and direct comparison with authentic samples of the dinitrophenylhydrazones prepared from the known unsaturated ketones (Table I). Furthermore, cleavage of the ketone derivatives with pyruvic acid–hydrogen bromide by a slight modification of the Mattox-Kendall procedure[11] led to the unsaturated ketones. As described in detail in the experimental section the water content of the pyruvic acid apparently plays an important role in this cleavage. The regeneration of the carbonyl compound was nearly complete in the case of the Δ^4- and Δ^1-3-ketones (IIa and IVa), but only partial with Δ^1-2-bromo compounds VIa (up to 30% of ketone, the remainder being recovered as hydrazone).

The remarkable fact about the dehydrobromination is that it is complete in a matter of minutes in acid solution in the presence of one mole of the very weak base[12] dinitrophenylhydrazine, while refluxing for fifteen minutes to four hours is required with a large excess of comparatively strong bases such as collidine or pyridine. Initial attack on the bromine by dinitrophenylhydrazine is quite unlikely, since under the same conditions (1.0–1.2 moles of base in glacial acetic acid) no comparable reaction is observed with benzylamine, aniline, ethylaniline or collidine. On the other hand, phenylhydrazine, α-(2,4-dinitrophenyl)-α-methylhydrazine, hydroxylamine and semicarbazide[13] produced essentially the same results as dinitrophenylhydrazine. Initial formation of a hydrazone of the bromo ketone as intimated by Mattox and Kendall[11] leaves no reasonable path for the elimination of hydrogen bromide, unless it is assumed that the bromine atom is so labilized by hydrazone formation as to be lost on warming for a few minutes in acetic acid.

It is suggested that the results are compatible with a reaction mechanism employing the concept of participation of neighboring groups in nucleophilic displacement reactions as developed by Winstein and co-workers in a brilliant series of papers,[14] and the reaction is depicted as proceeding as follows, a 2-bromo-3-keto*allo*steroid (III) being taken as an example



The initial attack of dinitrophenylhydrazine on the ketone III proceeds in the usual manner[15] to

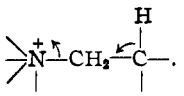
(12) See Davies, *J. Chem. Soc.*, 715 (1922) and Sidgwick, "Organic Chemistry of Nitrogen," Oxford, 1937, p. 395.

(13) All of the reagents (both successful and unsuccessful) were tried with 2-bromocholestanone. Phenylhydrazine and hydroxylamine hydrochloride did result in loss of hydrogen bromide but the product crystallized very poorly. Examples with the other two reagents are given in the experimental section. It should be noted however, that dinitrophenylhydrazine surpasses all reagents because of the ease with which the dinitrophenylhydrazones crystallize in the steroid series.

(14) See for instance Winstein, Grunwald, Buckles and Hanson, THIS JOURNAL, **70**, 816 (1948).

(15) Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 334.

yield intermediate A, in which the nitrogen atom with its available electron pair represents a powerful neighboring group, thus leading to expulsion of a bromide ion and the formation of a cyclic imonium compound such as B, in which the carbonium ion C represents one of the extreme ionic forms contributing to the resonance hybrid. The hydroxy–imonium compound B is formally identical with quaternary ammonium salts in the Hofmann reaction where elimination of the β-hydrogen occurs as well as rupture of the N–C bond:



The mechanism of the Hofmann elimination is only incompletely understood[16] and the reaction is therefore written in the non-committal form B, even though it may proceed through the carbonium ion C. The strain of the positively charged three-membered ring and the tendency toward dehydration constitute a strong driving force in the expulsion of the proton and fission of the N–C bond with the resulting formation of the final product IVb. Furthermore it is necessary to ascribe an important function (possibly due to momentary formation of a second positive charge) to the adjoining second nitrogen atom in the Hofmann-type elimination which proceeds under such mild conditions in an acid medium, in contrast to the usual basic thermal treatment. This last assumption is a necessary corollary to the imonium ring mechanism, since otherwise primary and secondary amines, *e. g.*, ethylaniline or benzylamine, should also be effective in the dehydrobromination.[17]

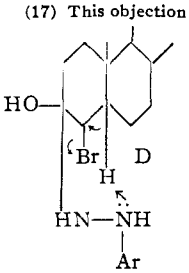
At this point it is necessary to consider the stereochemistry of the bromo compounds. As mentioned in the first paragraph, there exists some evidence[4] that 3-ketosteroids of the "normal" series on bromination often yield both stereoisomeric 4-bromo compounds, since only one form can be dehydrobrominated with ease. In the case of 2-bromo-3-keto*allo*steroids (III) such evidence is not available since *trans* elimination of hydrogen bromide is possible with either isomer. However, recent work[18] based on molecular rotation differences points toward the presence of both isomers in this series. On that basis the two bromo compounds (III, R = C_8H_{17}, and R = $COOCH_3$) represent different isomers at C-2 and yet both reacted equally fast with dinitrophenylhydrazine. Since the formation of the cyclic imonium compound B would require a *trans* relationship between the bromine atom and the dinitrophenylhydrazyl radical as in A in order to allow for rearward attack, it is necessary to postulate that in the case of the 4-bromo-normal (I) and 2-bromo-*allo* compounds (III) either the entrance of the dinitrophenylhydrazine molecule is always directed by steric factors of the steroid molecule in such a manner that a *trans* relation (A) is present, or more likely that a rapid equilibrium exists in which only one form (*trans*) reacts.

In the case of the 2,4-dibromo-3-keto*allo*steroids (VII), it is known that the 4-bromine atom has the (β)-configuration, since it can be eliminated with collidine[7,8] in less than one minute with the formation of the unsaturated bromo ketone IX. The configuration of the 2-bromine atom in VII is not known, but it is likely, again on the basis of rotation measurements,[18] that it has one and the same configuration in all of the *allo* compounds studied so far. It is conceivable that if a cyclic imonium ring mechanism operates, both bromines could be removed by one mole of dinitrophenylhydrazine, since a second ring could be formed after elimination of the first bromine atom, unless (a) dehydration to the hydrazone is an essential factor in that step, (b) both bromine atoms do not possess the same stereochemical configuration and (c) the hydroxyl group introduces steric hindrance. When the reaction was applied to 2,4-dibromo ketones (VII, R = C_8H_{17}, $COOCH_3$, $OCOC_6H_{11}$), a mixture was obtained, which by analysis still contained one mole of bromine, but which seemed to contain at least some of the $\Delta^{1,4}$-dien-3-one hydrazone VIIIb on the basis of the ultraviolet absorption spectra (Fig. 1). The hydrazone mixture could not be cleaved with pyruvic acid which was in agreement with experiments carried out on the hydrazones prepared from authentic dienones, and the results, therefore, are not conclusive.[19]

In order to elucidate further the action of dinitrophenylhydrazine on the 2,4-dibromo ketones VII, the 2-bromo-Δ^4-3-ketones (IX) were investigated since they might be intermediates in the reaction of VII. The surprising observation was made that those ketones reacted with dinitro-

(16) *Cf.* Hughes and Ingold, *Trans. Faraday Soc.*, **37**, 657 (1941).

(17) This objection has been brought up during a discussion at the University of California at Los Angeles. Drs. C. F. Koelsch and S. Winstein suggested as a possible alternative a mechanism, D, in which the α-nitrogen atom (which appears to be essential from our observations) assumes the dominant role by removing the proton on the β-carbon atom, the remaining steps being unexceptional. Such a suggestion, however, involves certain difficulties in the reaction of the Δ^4-2-bromoketones (IX) leading to Xb, which incidentally could also be accomplished with semicarbazide hydrochloride.



(18) Djerassi, *J. Org. Chem.*, **12**, 823 (1947).

(19) Recently, an article appeared [Klein, Weiner and Gordon, *Anal. Chem.*, **20**, 174 (1948)] in which it was postulated that α,β-unsaturated steroid ketones such as progesterone reacted with dinitrophenylhydrazine to form a pyrazoline derivative, since the product could not be split with acid. It has since been shown [Djerassi, *Anal. Chem.*, **20**, 880 (1948)] that the product is in fact the bis-dinitrophenylhydrazone since it led to progesterone in 60% yield on cleavage with pyruvic acid. In view of the resistance of $\Delta^{1,4}$-dien-3-one hydrazones (VIIIb) even toward pyruvic acid, it might be suggested that they represent pyrazoline derivatives (according to ref. 26, pyrazolines are not affected by pyruvic acid), but the absorption spectra speak against such a supposition. By analogy to the ultraviolet absorption spectra of other steroid hydrazones (ref. 20), such pyrazolines would be expected to have maxima below 390 mμ, rather than at 402 mμ as observed for the reaction product.

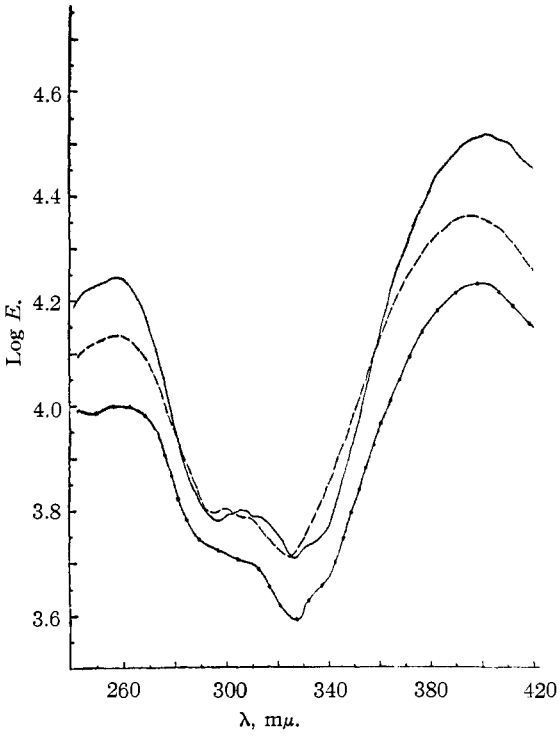
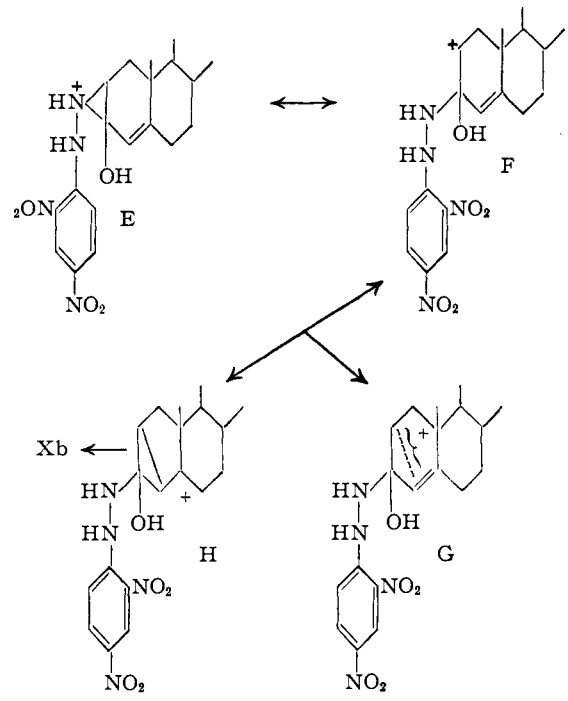
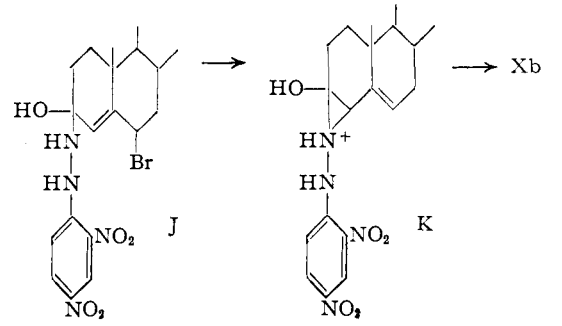


Fig. 1.—Ultraviolet absorption spectra in chloroform solution: — 1,4-androstadien-17-ol-3-one 17-hexahydrobenzoate dinitrophenylhydrazone; - - - - - product from reaction of 2,4-dibromoandrostan-17-ol-3-one 17-hexahydrobenzoate and 2,4-dinitrophenylhydrazine; —·—·— product from reaction of methyl 2,4-dibromo-3-keto*allo*etiocholanate and 2,4-dinitrophenylhydrazine.

phenylhydrazine to afford in about 45% yield the hydrazones of the corresponding $\Delta^{4,6}$-dien-3-one (Xb, R = C_8H_{17}, $COOCH_3$). The structure of the products was established by direct comparison with authentic samples (Table I) prepared from the known $\Delta^{4,6}$-dien-3-ones (Xa) as well as by their characteristic ultraviolet absorption spectra.[20] As was to be expected, the dinitrophenylhydrazones Xb were cleaved very incompletely with pyruvic acid but enough of the ketone was isolated to permit identification as the dienone Xa (m. p. and characteristic u. v. maximum at 282.5 mu). On applying the imonium-ring mechanism to this reaction, it is suggested that the primary intermediate is again a cyclic compound such as E. In this instance, the contributing carbonium ion form F, in contrast to C which lacks the double bond, is further stabilized by forms such as the hybrid carbonium ion G or the rearranged carbonium ion H. Forms F, G and H are somewhat comparable to the intermediates postulated in the *i*-steroid rearrangement.[21] It is realized that in this instance it is necessary to postulate a shift of hydrogen to C-2 in order to accommodate for structure Xb and the sequence of reactions from H leading to Xb is not depicted. Various explanations are possible for providing this shift, particularly since a proton donor is available in the solvent. It should be noted that if an alternate mechanism is considered in which the α-nitrogen atom is the essential factor,[17] it would be necessary to assume first that the bromine atom rearranged either to position 4 or 6, which could not be demonstrated (see experimental section), although it is conceivable that a very small amount of rearrangement product at any one time would be sufficient to drive the reaction to completion.



In view of the formation of the $\Delta^{4,6}$-derivative Xb from the Δ^4-2-bromoketone IX it was of considerable interest to investigate the behavior of the Δ^4-6-bromo-3-ketones XI (R = $COOCH_3$, $OCOC_6H_5$), where the bromine atom is not adjacent to the carbonyl group. As with the 2-bromo derivatives IX, the 6-bromo isomers XI also yielded the dinitrophenylhydrazones Xb although in appreciably higher yield. It is quite likely that in this instance, the primary reaction product J,

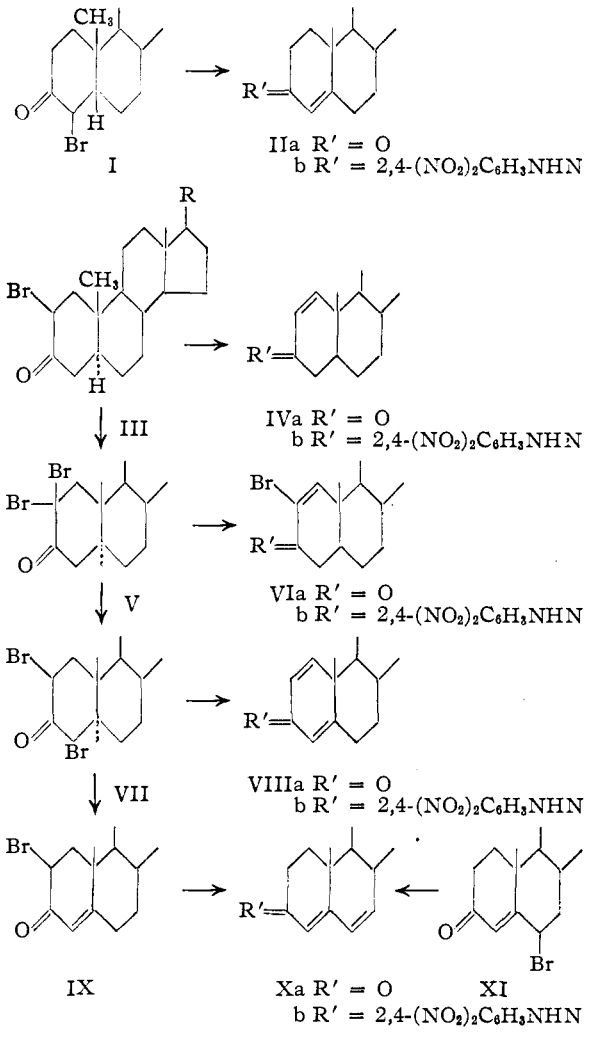


(20) Djerassi and Ryan, THIS JOURNAL, **71**, 1000 (1949).

(21) Winstein and Adams, *ibid.*, **70**, 838 (1948); Dodson and Riegel *J. Org. Chem.*, **13**, 424 (1948).

representing a particularly labile allylic system, undergoes simultaneous attack on position 4 by the nitrogen atom and shift of the double bond,[22] thus leading directly to the imonium compound K.

In conclusion, it can be stated that the Mattox–Kendall reaction[11] is useful for the dehydrobromination of 2-bromo-3-keto*allo*steroids and for 4-bromo-3-ketones of the normal series, particularly when employed on a small scale. The reaction has no practical applications in the other cases cited, unless a procedure is discovered for cleaving the dinitrophenylhydrazones of such poly-unsaturated ketones as the $\Delta^{4,6}$-dienones. It should be of interest to investigate this dehydrobromination procedure in simpler ketones other than steroids, where complicated steric factors do not enter and which may thus shed more light on the reaction mechanism.



Acknowledgment.—The author would like to express his gratitude to Dr. Gilbert Stork of Harvard University for the numerous stimulating discussions on the subject of this paper; furthermore, he is indebted to Dr. W. E. Doering of Columbia University for comments and to the Misses Helen Dudek and Jean Rogers for technical assistance. Since the completion of this work, the author has had the benefit of a critical discussion regarding the reaction mechanism with members of the summer staff of the University of California at Los Angeles, and he is thankful to Dr. S. Winstein for providing that opportunity.

(22) *Cf.* Roberts, Young and Winstein, THIS JOURNAL, **64**, 2157 (1942).

Experimental[23]

General Procedure for Reaction of Steroid Ketones with 2,4-Dinitrophenylhydrazine.—The following method was used for both the dehydrobromination of steroidal bromo ketones as well as for the preparation of comparison samples of dinitrophenylhydrazones from authentic saturated and unsaturated 3-ketosteroids.

Approximately 200 mg. of steroid was dissolved in 5–10 cc. of warm glacial acetic acid (depending on the solubility of the ketone) in an open Erlenmeyer flask while passing a slow current of nitrogen over the surface of the solution, 1.1 moles of dinitrophenylhydrazine was added, and the solution was heated on the hot-plate for five minutes in the open flask without condenser. In those instances where the hydrazone precipitated immediately, the heating time was reduced to three minutes. In practically all cases the hydrazone crystallized on cooling. Recrystallization was best effected from a mixture of ethanol and chloroform (all of the hydrazones were very soluble in chloroform) and all reactions involving solutions of hydrazone were carried out in an atmosphere of nitrogen. Experiments ranging from 25 mg. to 1.2 g. of steroid gave equally good results and required no modification except for variations in the amount of acetic acid. Remarks as to the applicability of this procedure to the dehydrobromination of the various classes of steroidal bromo ketones are given below in appropriate sections together with the physical constants of the products. In Table I are summarized the constants of some previously undescribed dinitrophenylhydrazones of both saturated and unsaturated steroid ketones which were prepared for one or more of the following purposes: Determination of the ultraviolet absorption spectra which are reported elsewhere,[20] preparation of authentic samples for comparison with the products arising from the dehydrobromination, and as model substances for determining conditions for the regeneration of the carbonyl compound from the hydrazone.

General Procedure for the Cleavage of Steroidal Dinitrophenylhydrazones.—The dehydrobromination procedure of Mattox and Kendall[11] is useful only if the unsaturated carbonyl compound can be regenerated from its dinitrophenylhydrazone. These workers indicated that the cleavage of the dinitrophenylhydrazones of Δ^4-3-ketosteroids could be accomplished in nearly quantitative yield by warming for two and one-half hours at 45° in 20 cc. of chloroform, 30 cc. of pyruvic acid and 2.2 cc. of 2.3 *N* hydrogen bromide solution (in acetic acid), but they neglected to state the quantity of hydrazone which could thus be cleaved. Using the dinitrophenylhydrazones of Δ^1- and Δ^4-cholesten-3-one our initial experiments to confirm those results failed completely. The following variations also resulted in partial or complete recovery of the hydrazone: Refluxing the reaction mixture (containing hydrazone, pyruvic acid and chloroform) with either 4 *N* hydrogen bromide–acetic acid or hydrogen bromide–acetic acid–

(23) All melting points are uncorrected. The microanalyses were carried out by Mr. Joseph F. Alicino, Metuchen, N. J., and Mr. George L. Stragand, Microchemical Laboratory, University of Pittsburgh.

TABLE I

AUTHENTIC SAMPLES OF DINITROPHENYLHYDRAZONES OF SOME STEROID KETONES[a]

Dinitrophenylhydrazone of	M. p., °C., (dec.)	Formula	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
Cholestan-3-one	228–230	$C_{33}H_{50}O_4N_4$	69.93	8.89	9.89	69.74	8.99	9.70
2-Acetoxycholestan-3-one[b]	173–175	$C_{35}H_{52}O_6N_4$			8.97			8.60
Dihydrotestosterone hexahydrobenzoate	206–208	$C_{32}H_{44}O_6N_4$	66.17	7.64	9.65	66.61	7.77	9.25
Methyl 3-ketoetio*allo*cholanate	225–228	$C_{27}H_{36}O_6N_4$	63.26	7.08	10.93	63.11	7.46	10.84
Methyl Δ^1-3-ketoetio*allo*cholenate[c]	270–272	$C_{27}H_{34}O_6N_4$	63.51	6.71	10.97	63.36	6.46	11.31
Δ^1-Cholesten-3-one	218–220	$C_{33}H_{48}O_4N_4$	70.18	8.57	9.92	69.99	8.79	9.81
Δ^4-Cholesten-3-one	232–235	$C_{33}H_{48}O_4N_4$	70.18	8.57	9.92	69.76	8.63	9.52
Δ^1-2-Bromocholesten-3-one[d]	265–267	$C_{33}H_{47}O_4BrN_4$	61.57	7.36	8.71	61.36	7.57	8.97
Δ^1-2-Bromoandrosten-17-ol-3-one 17-hexahydrobenzoate[e]	267–270	$C_{32}H_{41}O_6BrN_4$	58.44	6.29	8.52	58.37	6.45	8.55
$\Delta^{4,6}$-Cholestadien-3-one	227–229	$C_{33}H_{46}O_4N_4$	70.43	8.24	9.96	70.05	8.25	10.24
Methyl $\Delta^{4,6}$-3-ketoetiocholadienate	254–256	$C_{27}H_{32}O_6N_4$	63.76	6.34	11.02	63.59	6.32	11.05
Methyl $\Delta^{1,4}$-3-ketoetiocholadienate	180–183	$C_{27}H_{32}O_6N_4$	63.76	6.34	11.02	64.18	6.48	11.52
$\Delta^{1,4}$-Cholestadien-3-one	183–184	$C_{33}H_{46}O_4N_4$	70.43	8.24	9.96	70.50	8.60	10.12
$\Delta^{1,4}$-Androstadien-17-ol-3-one 17-hexahydrobenzoate	263–264	$C_{32}H_{49}O_6N_4$	66.64	6.99	9.72	66.80	6.92	9.56

[a] With one exception (preparation given in this paper) all of the ketones have been described in the literature. [b] Acetyl, calcd.: 6.89; found: 7.29. [c] Methoxyl, calcd.: 6.08; found: 5.85. [d] Bromine, calcd.: 12.42; found: 12.45. [e] Bromine, calcd.: 12.15; found: 11.92.

water,[24] using Hershberg's[25] pyruvic acid cleavage for semicarbazones, or that of Anchel and Schoenheimer[26] for splitting *p*-carboxyphenylhydrazones. Similarly, refluxing with pyruvic acid or biacetyl[27] alone resulted in recovery of the hydrazone. Finally, it was noted that the water content of the pyruvic acid greatly influenced the extent of cleavage and this unquestionably was the reason why Mattox and Kendall's procedure[11] could not be repeated originally. The following method gave fairly reproducible results:

A solution of 100 mg. of steroid dinitrophenylhydrazone in 5 cc. of chloroform is treated in a stoppered flask (air displaced by nitrogen) at 50–60° with 7.5 cc. of commercial pyruvic acid (83–85%) and 0.6 cc. of approximately 4 *N* anhydrous hydrogen bromide in acetic acid for three hours. It appears that pyruvic acid of neutral equivalent 105 or higher yields two layers under the conditions specified and hence continuous shaking is essential for cleavage. Pyruvic acid of neutral equivalent 102 or less gives a homogeneous solution, thus not requiring any shaking, and the yield of ketone seems to be higher. The completion of the reaction is usually indicated by appreciable lightening of the color of the solution. After dilution with chloroform and extraction with carbonate solution, the organic layer is dried and if the residue is still colored, it is recrystallized preferably from ethanol (using Norit), since any unchanged hydrazone is practically insoluble in that solvent.

This procedure has given satisfactory results in the cleavage of hydrazones of saturated as well as Δ^1- and Δ^4-unsaturated 3-ketosteroids,[28] where 60–70% of the pure ketone could be isolated. Losses in the purification of the ketone on such a small scale may be responsible for the lower yield as compared to that reported by Mattox and Kendall.[11] With Δ^1-2-bromo-3-keto derivatives, the reaction proceeded to the extent of 20–30%, while in the case of the hydrazones of $\Delta^{4,6}$-dien-3-ones only about 5–10% of the ketone could be isolated, the rest being recovered as unchanged hydrazone. The derivatives of $\Delta^{1,4}$-dien-3-ones could not be split at all.[19] The α-(dinitrophenyl)-α-methylhydrazone of even a singly unsaturated ketone (such as Δ^1-cholestenone) also were unaffected under those conditions.

Preparation of Bromo Ketones.—With a few exceptions the synthesis of the bromo ketones used below for the dehydrobrominations has been described in papers from this or other laboratories.[2,6,8,29,30] The following either are new compounds or require comment.

Δ^4-2-Bromocholesten-3-one (IX, R = C_8H_{17}).—By analogy to related 2,4-dibromo ketones[7,8] 4 g. of 2,4-dibromocholestan-3-one[29] was refluxed for forty seconds with 18 cc. of redistilled collidine, which resulted in the loss of 1.2 moles of hydrogen bromide on the basis of collidine hydrobromide isolated. After working up as usual and recrystallizing from ethanol, there was obtained 1.17 g. (34%) of colorless, long needles melting at 117–119°, $[\alpha]^{29}D$ + 81° (chloroform), maximum at 243 mu, log *E* 4.15 (ethanol).

Anal. Calcd. for $C_{27}H_{44}OBr$: C, 69.80; H, 9.55; Br, 17.21. Found: C, 69.81; H, 9.22; Br, 17.05.

The structure of the compound was confirmed by further dehydrobromination to $\Delta^{1,4}$-cholestadien-3-one and dienone-phenol rearrangement of the latter to the corresponding 1-methylphenol.[29]

Methyl Δ^4-6-Bromo-3-ketoetiocholenate (XI, R = $COOCH_3$).—The method of Ruzicka and Bosshard[30] for 6-bromotestosterone benzoate was applied to methyl Δ^5-3-hydroxyetiocholenate by adding one mole of bromine, oxidizing with chromic anhydride and dehydrobrominating the crude 5,6-dibromo-3-ketone with anhydrous sodium acetate in ethanol. After recrystallization from hexane–acetone, there was obtained 59% of the desired unsaturated bromo ketone with m. p. 136–137°, $[\alpha]^{26}D$ + 30° (chloroform). Reich and Lardon[31] prepared the compound by the action of N-bromoacetamide on the enol acetate of the corresponding Δ^4-3-ketone and reported m. p. 139–140°. The structure of the bromo ketone was proved by collidine dehydrobromination to methyl $\Delta^{4,6}$-3-ketoetiocholadienate with its characteristic maximum at 282.5 mμ, and which gave no depression in m. p. on admixture with a sample prepared by the following method.

(24) Johnson, Petersen and Schneider, THIS JOURNAL, **69**, 75 (1947), described a mixture of acetic acid, 48% hydrobromic acid and water from which the hydrogen bromide was not lost on heating which was initially believed to be the reason why cleavage was unsuccessful on refluxing with anhydrous hydrogen bromide–acetic acid.

(25) Hershberg, *J. Org. Chem.*, **13**, 542 (1948).

(26) Anchel and Schoenheimer, *J. Biol. Chem.*, **114**, 539 (1936).

(27) Strain, THIS JOURNAL, **57**, 758 (1935).

(28) Even a 3,20-bis-dinitrophenylhydrazone (see ref. 19 for cleavage of progesterone bis-dinitrophenylhydrazone) can be split under those conditions.

(29) Wilds and Djerassi, THIS JOURNAL, **68**, 1712 (1946).

(30) Ruzicka and Bosshard, *Helv. Chim. Acta*, **20**, 328 (1937).

(31) Reich and Lardon, *ibid.*, **29**, 671 (1946).

Methyl $\Delta^{4,6}$-3-Ketoetiocholadienate (Xa, R = $COOCH_3$).—This previously unknown dienone was needed for comparison with the above sample prepared by dehydrobromination of the Δ^4-6-bromo-3-ketone as well as for conversion to an authentic sample of dinitrophenylhydrazone. Wettstein's modification[32] of the Oppenauer oxidation presented a convenient one-step synthesis. Two grams of methyl Δ^5-3-hydroxyetiocholenate was oxidized with quinone exactly as described for cholesterol[29] except that commercial aluminum isopropylate was substituted for the *t*-butoxide.[33] After chromatographing the crude, crystalline residue and recrystallizing from methanol, there was obtained 0.81 g. (41%) of colorless rosettes of prismatic needles with m. p. 165–165.5°, $[\alpha]^{26}D$ + 125° (chloroform), max. at 282.5 mμ (log *E* 4.49), and min. at 235 mμ., (log *E* 3.46 in ethanol).

Anal. Calcd. for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59; methoxyl, 9.45. Found: C, 76.75; H, 8.48; methoxyl, 9.67.

Dehydrobrominations with 2,4-Dinitrophenylhydrazine

(a) **2-Bromo-3-keto*allo*steroids (III).**—The dehydrobromination of 2-bromocholestanone and methyl 2-bromo-3-ketoetio*allo*cholanate led in 80–90% yield to the hydrazones of the corresponding Δ^1-3-ketones. These were found to be identical (m. p., u. v. spectrum, analysis) with authentic samples described in Table I and were further identified by pyruvic acid cleavage to the known[8] unsaturated ketones. 2-Bromoandrostan-17-ol-3-one 17-hexahydrobenzoate gave 83% of the corresponding Δ^1-unsaturated dinitrophenylhydrazone, m. p. 237–239° (dec.), the analysis of which is reported since an authentic sample was not available.

Anal. Calcd. for $C_{32}H_{42}O_6N_4$: C, 66.41; H, 7.32; N, 9.68. Found: C, 66.69; H, 7.68; N, 9.93.

It is interesting to note that the last mentioned two 2-bromo compounds are believed[18] to possess a different configuration of the bromine atom as compared to 2-bromocholestanone, but that no difference in the rate of dehydrobromination was observed.

(b) **2,2-Dibromo-3-keto*allo*steroids (V).**—The general procedure for dehydrobromination was varied slightly in that the dinitrophenylhydrazine was dissolved in hot glacial acetic acid first and the dibromo ketones were then added, since it has been shown previously[8] that those ketones rearrange in a few minutes on heating in acetic acid to the 2,4-isomers. When applied to 2,2-dibromocholestan-3-one and 2,2-dibromoandrostan-17-ol-3-one 17-hexahydrobenzoate, 58–62% of the corresponding Δ^1-2-bromo hydrazones was isolated on cooling. These products were identical with samples prepared from the known Δ^1-2-bromo-ketones (*cf.* Table I).

(c) **4-Bromo-3-ketosteroids (I).**—These have already been investigated by Mattox and Kendall[11] and only one additional example was examined in order to demonstrate the applicability of our reaction conditions to such ketones. Methyl 3-keto-4-bromo-12-hydroxycholanate afforded 87% of methyl Δ^4-3-keto-12-hydroxycholenate dinitrophenylhydrazone melting at 240–242° (dec.) after recrystallization from ethanol–chloroform. The ultraviolet absorption spectrum has already been reported.[20]

Anal. Calcd. for $C_{31}H_{42}O_7N_4$: C, 63.90; H, 7.27; N, 9.58. Found: C, 63.54; H, 7.35; N, 9.39.

Pyruvic acid cleavage of 70 mg. of this dinitrophenylhydrazone gave 35 mg. (72%) of methyl Δ^4-3-keto-12-hydroxycholenate with m. p. 147–149°, $[\alpha]^{27}D$ + 78° (acetone), identical with a sample prepared by another method.[34]

(d) **2-Bromo-Δ^4-3-ketosteroids (IX).**—Bromo ketones of this type have been reported[7] to be rearranged in the presence of hydrogen bromide to presumably the isomeric 6-bromo isomers. Since in the present dehydrobromination procedure, the ketone is heated first in acetic acid before dinitrophenylhydrazine is added, it was necessary to show that no rearrangement occurred during the initial heating period. This was tested in the case of methyl Δ^4-2-bromo-3-ketoetiocholenate[35] which was recovered unchanged (melting point, bromine analysis, rotation) on heating for five minutes in glacial acetic acid. When this bromo ketone or 2-bromo-Δ^4-cholesten-3-one was heated with dinitrophenylhydrazine in the usual manner, 44–46% of the dinitrophenylhydrazone (Xb) of the corresponding $\Delta^{4,6}$-dien-3-one was obtained. These compounds exhibited a shiny-red color and showed typical maxima at 309 mμ and 404 mμ as discussed elsewhere.[20] Furthermore, they were identical with samples prepared from authentic dienones (Table I) and on cleavage with pyruvic acid, 100 mg. of $\Delta^{4,6}$-cholestadien-3-one dinitrophenylhydrazone afforded 5 mg. of the ketone,[29] characterized by its selective absorption at 282.5 mμ and melting point. It is interesting to note that in the dehydrobomination of these compounds or the isomeric 6-bromo derivatives described below, occasionally on cooling the acetic acid solution yellowish-orange colored crystals appeared instead of bright red ones which, however, turned reddish on filtering and assumed the usual color on subsequent recrystallization. No obvious explanation seems evident for this behavior.

(e) **Δ^4-6-Bromo-3-ketosteroids (XI).**—Methyl Δ^4-6-bromo-3-ketoetiocholenate and 6-bromotestosterone benzoate[30] on dehydrobromination with dinitrophenylhydrazine behaved exactly as the above mentioned 4-bromo isomers, except that the yield of $\Delta^{4,6}$-dienone hydrazone (Xb) was appreciably higher (69–71%). 6-Dehydrotestosterone benzoate dinitrophenylhydrazone melted at 252–254° and showed ultraviolet absorption maxima at 309 mμ (log *E* 4.10) and 404 mμ (log *E* 4.49 in chloroform), typical of this type of conjugated system.[20]

Anal. Calcd. for $C_{32}H_{34}O_6N_4$: C, 67.35; H, 6.01; N, 9.82. Found: C, 66.94; H, 5.88; N, 9.88.

(f) **2,4-Dibromo-3-ketoallosteroids (VII).**—The behavior of 2,4-dibromocholestanone, methyl 2,4-dibromo-3-ketoetio*allo*cholanate and 2,4-dibromoandrostan-17-ol-3-one 17-hexahydrobenzoate with one mole of dinitrophenylhydrazine in glacial acetic acid was investigated and in each case it was necessary to add water to the reaction mixture in order to precipitate the products. They evidently consisted of a mixture which was not amenable to effective separation by recrystallization and which could not be cleaved with pyruvic acid, but which by analysis still contained approximately one bromine atom. On the basis of the ultraviolet absorption spectra (Fig. 1), it seems likely that some of the desired $\Delta^{1,4}$-dien-3-one dinitrophenylhydrazone was formed.

Reaction of Steroid Ketones with α-(2,4-Dinitrophenyl)-α-methylhydrazine.[36]—The reactions with this reagent were carried out in glacial acetic acid as described for dinitrophenylhydrazine. Unfortunately, the resulting hydrazones crystallized very poorly in nearly all cases (gelatinous solutions) and with the exception of cholestanone, no sharp melting points were obtained. No definite product could be isolated from the reaction of this reagent with Δ^4-cholesten-3-one or methyl Δ^4-2-bromo-3-ketoetiocholenate. It has been pointed out[20] that those derivatives exhibit an interesting shift in the ultraviolet absorption maxima.

Cholestanone α-(2,4-dinitrophenyl)-α-methylhydrazone was obtained as a yellow solid with m. p. 203–205° which crystallized directly from the reaction mixture.

Anal. Calcd. for $C_{34}H_{52}O_4N_4$: C, 70.31; H, 9.03; N, 9.65. Found: C, 69.99; H, 8.90; N, 9.58.

Δ^1-Cholestenone α-(2,4-dinitrophenyl)-α-methylhydrazone was isolated in 66% yield as a greenish-yellow solid on diluting the reaction mixture from the dehydrobromina-

(32) Wettstein, *Helv. Chim. Acta*, **23**, 388 (1940).

(33) A trial run with cholesterol using the earlier conditions (ref. 29), but employing the more readily available aluminum isopropylate gave the same yield of $\Delta^{4,6}$-cholestadien-3-one.

(34) Djerassi and Scholz, *Experientia*, **3**, 107 (1947).

(35) The melting point of this compound was previously reported (ref. 8) as 162.5–163.5°. On repeating the synthesis, it was possible to raise the melting point to 167–167.5°, without, however, changing the rotation.

(36) Blanksma and Wackers, *Rec. trav. chim.*, **55**, 655 (1936).

tion of 2-bromocholestanone with this reagent, and recrystallizing from ethanol–chloroform (gelatinous solution); m. p. 166–177° (dec.).

Anal. Calcd. for $C_{34}H_{50}O_4N_4$: C, 70.55; H, 8.71; N, 9.68. Found: C, 70.23; H, 8.55; N, 9.57.

Methyl 2-bromo-3-ketoetio*allo*cholanate as well as 2,2-dibromocholestanone could be dehydrobrominated in a similar manner, but the products gave analytical figures which were always 2% too low in carbon content.

Reaction of 2-Bromocholestanone with Semicarbazide Hydrochloride.—A solution of 230 mg. of 2-bromocholestanone in 5 cc. of glacial acetic acid was heated with 60 mg. of finely crushed semicarbazide hydrochloride for six minutes. After dilution with water and recrystallization from ethanol–chloroform, there was obtained 170 mg. (78%) of Δ^1-cholestenone semicarbazone with m. p. 233–235°, undepressed on admixture with an authentic specimen; the derivative showed the typical maximum at 266 mμ, log *E* 4.41 (chloroform). The product gave a negative Beilstein test and was cleaved almost instantaneously with dioxane–43% sulfuric acid to yield 86% of Δ^1-cholestenone. It should be noted that while dinitrophenylhydrazones are usually isolated and purified more readily than the corresponding semicarbazones, the latter are very easily split into their components.[25]

Summary

The dehydrobromination of steroidal bromo ketones with dinitrophenylhydrazine, first discovered by Mattox and Kendall[11] in the case of 4-bromo-3-ketosteroids, has been examined critically. In addition to the 4-bromo ketones, the method is applicable to the dehydrobromination of 2-bromo- and 2,2-dibromo-3-keto*allo*steroids as well as 2-bromo- and 6-bromo-Δ^4-3-ketosteroids. The last two compounds both yield the hydrazone of the $\Delta^{4,6}$-dien-3-one. A mechanism for the reaction is suggested which employs a cyclic imonium compound as the key intermediate.

The regeneration of the unsaturated carbonyl compounds from the dinitrophenylhydrazones was examined and was found to be feasible from a preparative standpoint only in the case of the Δ^1- and Δ^4-3-ketones, thus imposing somewhat of a limitation on the Mattox–Kendall reaction.

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Synthesis of 5,5′-Dibromosalicil and Related Compounds

BY JACOB FINKELSTEIN AND SEYMOUR M. LINDER

In reports distributed by the Office of The Publication Board, No. 20,466 (Bios Final Report No. 219) it was revealed that Kuhn, in 1943, at Heidelberg had prepared 2,2′-dihydroxy-5,5′-dibromobenzil (3065) and claimed it to be the first compound found to be effective against Rickettsia and Influenza virus strain A employing mice as test animals. The I.G. Laboratories at Elberfeld found this substance to be tolerated by man in ten-gram doses. However, they were disappointed in its action in bacterial infections when compared to the sulfa drugs of choice. The substance is extremely insoluble. Therefore, the solubilized form prepared in borax and soda was tested and showed a fatal dose in the mouse of 30 mg./kg. Nevertheless, the claim by another I.G. Laboratory of its activity against virus organisms served as a stimulus to prepare the substance for testing in our chemotherapeutic laboratories. Although we were unable to confirm Kuhn's chemotherapeutic findings for the compound, we continued the investigation by synthesizing closely related substances. However, none of the compounds prepared was active *in vivo*, but the chemistry involved is interesting and forms the subject of this paper.

The methods of synthesizing the dibromosalicil are rather straight-forward. Kuhn, *et al.*[1] employed two closely related procedures. In one they started with 1,1′-dimethoxybenzil which was previously reported by Schonberger and Kraemer[2] and Irvine.[3] The compound was smoothly demethylated by aluminum chloride and then brominated in acetic acid. The other method by Perkin[4] starts with 2-methoxy-5-bromobenzaldehyde undergoing the benzoin condensation to produce the bromomethoxybenzoin. Another synthesis was outlined in the Department of Commerce Publication paralleling the latter method starting with 2-methoxymethoxy-5-bromobenzaldehyde (I). This methoxymethoxy substituent is ultimately removed by extremely mild conditions to produce the dibromosalicil.

For our investigation, the last method discussed was selected and the compound at each intermediate stage isolated and chemotherapeutically tested. In extending the problem to the preparation of closely related compounds, different compounds from those expected were isolated in several instances.

The first compound in the series capable of variation, 2,2′-di-(methoxymethoxy)-5,5′-dibromobenzoin (II), was subjected to hydrolysis for two minutes to produce the 2,2′-dihydroxy derivative (III). However, under identical conditions and with the reaction time increased to thirty minutes, the expected phenolic compound was readily acetylated to form 2,2′-diacetoxy-5,5′-dibromobenzoin (IV). The structure of this compound was proven by a negative color reaction with ferric chloride and conversion to III by hydrolysis with 10% sodium hydroxide.

(1) Kuhn, *et al.*, *Ber.*, **76**, 900 (1943).
(2) Schonberger and Kraemer, *ibid.*, **55**, 1184 (1922).
(3) Irvine, *J. Chem. Soc.*, **79**, 670 (1901).
(4) Perkin, *Ann.*, **145**, 304 (1868).