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Hydrogen Bonding in 7-Ketocholesterol and a New Isomorph of 7-Ketocholesteryl Acetate

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In 1946 Furchgott, Rosenkrantz, and Shorr (*1*), employing infrared techniques, demonstrated that many steroids undergo hydrogen bonding in the solid state. This phenomenon has been observed, not only for the physiologically important steroids (*1, 2*), but also for ergostane compounds (*3*) and in the cholesterol series (*4*). In all cases one hydroxyl band occurred and the hydrogen bonding was evident because of displacement of the hydroxyl stretching vibrations from the unassociated wave number near 3640 cm^{-1} (wavelength, 2.75 μ) to wave numbers nearer 3330 cm^{-1} (wavelength, 3 μ).

During the infrared studies on the cholestane derivatives (*4*), it was observed that only 7-ketocholesterol gave rise to two absorption bands in the hydroxyl region. This occurred in the spectra of solid films, Nujol mulls, carbon disulfide (10 mg/ml, 1-mm cell) and carbon tetrachloride (5 mg/ml, 2-mm cell) solutions. In the solid state both bands were nearly of equal intensity, the sharper one occurring near 3545 cm^{-1} (wavelength 2.82 μ), while a broader one was located near 3280 cm^{-1} (wavelength 3.05 μ). In the solutions the usual increase in wave number owing to this state was observed, 3615 cm^{-1} (wavelength 2.77 μ) and 3417 cm^{-1} (wavelength 2.93 μ), respectively. No significant displacement of the ketone absorption occurred either in the solid state or in solution.

A doublet in the hydroxyl region was observed by Jones *et al.* (*2*) in the spectra of 17α-hydroxy-20-ketosteroids. Intramolecular hydrogen bonding was proposed as an explanation for the hydroxyl doublet, and it was reported that a threefold dilution in carbon tetrachloride did not alter the relative intensities of the two hydroxyl bands. Splitting of the normal 20-ketone absorption band was interpreted as indicative of an equilibrium between bonded and unbonded molecules. One other instance of the appearance of a doublet in the 3-μ region has been reported for cholestane-3β,5α-diol-6-one (*5*).

Dilution of a solute involved in hydrogen bonding is known to decrease the intensity of the associated absorption band and increase that of the higher frequency band. In the present study (*6*) a twofold or threefold dilution in carbon tetrachloride or carbon disulfide did not alter the relative intensities of the two hydroxyl bands. Before postulating hydrogen bonding for the origin of the 3-μ doublet, the possibility of tautomerism was examined by attempting to synthesize the enol acetate of 7-ketocholesterol. On refluxing 7-ketocholesteryl acetate with acetic anhydride and acetyl chloride, $\Delta^{3,5}$-cholestadiene-7-one (*7*) was obtained in 25-percent yield. The structure was established by infrared analysis, melting point 110° to 112°C, and elemental analysis: found C, 84.52; H, 10.90; calculated C, 84.75; H, 11.07. Treatment with acetic anhydride and sodium acetate or with acetic anhydride and pyridine resulted in formation of a dimorph (mp 163° to 164°C; found C, 78.57; H, 10.41; calculated C, 78.68; H, 10.47) of the starting material (mp 153° to 155°C). The lower melting modification could be transformed into the higher melting form by seeding its pyridine solution with crystals of the new isomorph. Both compounds gave identical infrared spectra in carbon disulfide solution (10 mg/ml; 1-mm cell; 12 C Perkin-Elmer infrared spectrometer).

Ultraviolet spectroscopic analysis was also employed in an attempt to estimate quantitatively any possible enol structure. If 7-ketocholesteryl acetate enolized, then either a $\Delta^{5,7}$-diene or a more stable $\Delta^{4,6}$-diene would arise. The absorption of the latter arrangement cannot be distinguished from the 240-mμ absorption of the parent molecule, but the $\Delta^{5,7}$-structure absorbs near 285 mμ. Therefore, although variation in the density at 240 mμ could not be interpreted, appearance of a maximum near 285 mμ could favor the formation of the $\Delta^{5,7}$-diene intermediate.

7-Ketocholesteryl acetate was studied in neutral (isooctane), acidic (acetic anhydride plus acetyl chloride) and basic (0.07*N* tetramethylammonium hydroxide in 90-percent ethanol) solutions [method of A. S. Meyer, personal communication] in a 1-cm cell at concentrations of 20 μg/ml. Either a Beckman DU or Cary 11 MS spectrophotometer was used for the determinations at zero, 4 and 24 hr.

No absorption occurred under neutral or acidic conditions, while a maximum near 285 mμ in the basic medium could account for approximately 20 percent intermediate. Whether this 285-mμ maximum is related to the $\Delta^{5,7}$-diene structure under the basic conditions used remains to be seen, but it should be recalled that enol acetate formation is catalized by acids.

Failure to prepare the enol acetate of 7-ketocholesterol by the usual chemical means in addition to the ultraviolet findings indicated that tautomerism did not significantly occur in this molecule. Therefore, the origin of the two absorption bands in the hydroxyl region have been assigned to an unusual tendency of intermolecular hydrogen bonding in 7-ketocholesterol. Indeed, this phenomenon may explain the difficulty in synthesizing the enol acetate of 7-ketocholesterol. Ad-

ditional evidence for the possible interference of hydrogen bonding with enolization was obtained from attempts to synthesize the enol acetate of cholestane-3β,5α-diol-6-one which has been shown to give a doublet in the 3-μ region (*5*). On refluxing this steroid with acetic anhydride and acetyl chloride, only the 3,5-diacetate was isolated in excellent yield (*8*).

Although variations in the carbonyl absorption did not occur, it is believed that a hydroxyl-ketone type bonding was prevalent. This was indicated by examination of the spectrum of 7-ketocholestanol which had only one absorption band in the 3-μ region (*4*) and suggested that the double bond in the unsaturated analog afforded a more rigid structure that favored bonding of the carbonyl group. As expected, the infrared spectrum of 7-ketocholesteryl acetate gave no absorption bands in the hydroxyl region, confirming that the 3β-hydroxyl group also was involved in the hydrogen bonding. Furthermore, spectroscopic examination of 7β-hydroxycholestenone (*9*) disclosed only one hydroxyl band and fortified the interpretation that 7-ketocholesterol had a more favorable steric arrangement for hydrogen bonding than compounds that have similar functional groups at other positions.

It is suggested that difficulty in synthesizing enol acetates be examined on the basis of the possibility and intensity of hydrogen bonding.

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Communications

Big Business and Research

Although I am loath to engage in public controversy, Philip Reichert's communication [*Science* **120**, 434 (1954)] requires an answer. Reichert emphasizes that the university professor has as his first function education rather than the performance of research. This definition is one with which others might disagree, since there is considerable support in the history of universities for their function as centers of learning in which education of disciples and free investigation of ideas or objective observations have equal importance.

The application of business methods to research has resulted in "a flood of immediately practical therapy" without any doubt. However, the great advance in basic knowledge upon which the practical applications must be based have rarely resulted from business methods in research. Business is fundamentally interested in profit, and therefore it is axiomatic that the research must be directed toward a profitable end. Even the "basic research" supported by business is of limited scope and usually is directed toward the solution of some project that has business interest. To any scientist who has dealt with the research performed in business organizations, or who has attempted to find money for basic research in grants from business organizations, the truth of my statements must be obvious. I base them upon my own experiences in this regard.

"The widespread distribution of information on new medical products" is a plague of the medical profession. The information is never unbiased (well, hardly ever!). Motivation need not be questioned. The results are evident in the pounds of "continuing postgraduate courses" that cross my desk daily. Several examples lie before me as I write, published by "ethical pharmaceutical" concerns, in which recommendations are made as though they were based upon incontrovertible fact, whereas these recommendations, in truth, either are directly contrary to the majority opinion among responsible investigators or are the subject of raging controversy.

"From the point of view of the patient—the average citizen—," he had better depend on physicians who form their opinions independently, from sources of information that are divorced from the immediate pressure of financial interest.

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6 October 1954.

In so far as R. W. Lippman's conclusions are based upon his own experiences, his conclusions are naturally valid for him. As a researcher who has crossed to the other side of the desk, I have during the last 10 years assisted in distributing many thousands of dollars in grants to approximately 20 first-line investigators. None of these men seem to be hampered by the fact that they are working for organizations that have a profit motive.

The difference between business research and university research is largely the pressure of the time element, and this usually means that the grants are