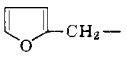
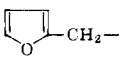
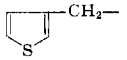
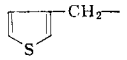
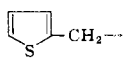
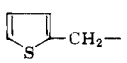
TABLE I

ANTIBACTERIAL ACTIVITY OF CEPHALOSPORANIC ACIDS AND PENICILLINS IN A GRADIENT PLATE TEST[8,a]

| Compound | Organism,[b] μg. to inhibit | | | | | | |
|---|---|---|---|---|---|---|---|
| | N9 | N10 | N26 | X26 | X68 | K1 | S1 |
| II, R = $C_6H_5CH_2$– | 32 | 48 | 42 | 109 | 42 | 13 | 44 |
| I, R = $C_6H_5CH_2$– | 33 | 37 | 24 | 2 | 15 | 27 | 27 |
| II, R = $C_6H_5OCH_2$– | 112 | 144 | 110 | 138 | >200 | >200 | 105 |
| I, R = $C_6H_5OCH_2$– | 107 | 132 | 101 | 7 | 110 | >200 | 102 |
| II, R =  | 31 | 59 | 41 | >200 | 84 | 13 | 40 |
| I, R =  | 15 | 36 | 10 | 7 | 8 | 11 | 17 |
| II, R =  | 15 | 33 | 25 | 105 | 38 | 10 | 26 |
| I, R =  | 31 | 33 | 17 | 2 | 7 | 20 | 8 |
| II, R =  | 28 | 46 | 38 | 122 | 56 | 10 | 39 |
| I, R =  | 11 | 15 | 8 | 6 | 5 | 8 | 11 |
| I, R = D-$HO_2CCH(NH_2)(CH_2)_3$ | 75 | 104 | 79 | 38 | 48 | 37 | 73 |

[a] Results of this type test should be interpreted on a comparative basis only and require use of an internal standard for accuracy. [b] N9 = *Shigella sp.*; N10, N26 = *E. coli*; X26, K1 = *Klebsiella sp.*; X68 = *Aerobacter sp.*; S1 = *Sh. sonnei*.

several Gram negative organisms.[8] In the group of penicillins (Table I) no increase in Gram negative activity was observed over that exhibited by benzylpenicillin.[9] By contrast, cephalosporins with either thiophene-2-acetyl or furan-2-acetyl sidechains at position 7 showed at least a threefold enhancement of activity over benzylpenicillin. Further, *all* of the cephalosporanic acids showed as good or better action than the corresponding penicillins. It appears, therefore, that greater potential for Gram negative activity resides in the cephalosporin structure.

The sodium salt of 7-(thiophene-2-acetamido)-cephalosporanic acid, which has been given the generic name cephalothin, has received extensive clinical evaluation as a broad spectrum antibiotic. Results of this work will be reported later.

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RECEIVED AUGUST 1, 1962

(8) C. W. Godzeski, C. Brown, D. E. Pavey and J. McGowan in "Antimicrobial Agents and Chemotherapy—1961," Amer. Soc. for Microbiology, 1962, p. 547. See also C. W. Godzeski, G. Brier and D. E. Pavey, in preparation.

(9) A penicillin (II, R = D-$C_6H_5CH(NH_2)$ has been reported to have greater Gram negative activity than benzylpenicillin but to be ineffective vs. penicillin resistant staphylococci. See G. N. Rollinson and S. Stevens, *Brit. Med. Jour.*, (2), 191 (1961). We have prepared the analogous cephalosporanic acid (I, R = D-$C_6H_5CH(NH_2)$-) and find that it has substantial broad spectrum activity. However, the compound decomposed rapidly in aqueous solution so that quantitative comparisons of activity were unreliable.

## CONJUGATE ANION FORMATION AND ALKYLATION OF α,β-UNSATURATED KETONES[1]

*Sir:*

Sodium and potassium salts of tertiary alcohols are efficient bases for the alkylation of α,β-unsaturated ketones in the α-position.[2] The system methyl iodide–potassium *t*-butoxide *t*-butyl alcohol, has been utilized extensively for the conversion of $\Delta^4$-3-keto steroids (I),[3] as well as simple bicyclic derivatives,[4] into 4,4-dimethyl-$\Delta^5$-3-ketones (VI), but certain aspects of this reaction are poorly understood. Alkylation even with a limited amount of base and alkyl halide leads to the 4,4-dimethyl compound (VII) as the major product and the 4-monomethyl-$\Delta^4$-3-ketone (VI) as a minor product, indicating that the second alkylation step and/or tertiary carbanion formation proceeds more rapidly than the first alkylation step and/or secondary carbanion formation. Under special reaction conditions (slow addition of 1.2 equiv. of methyl iodide to the steroid and 1.5 equiv. of potassium *t*-butoxide in boiling *t*-butyl alcohol), Atwater[5] found that product formation can be reversed with monomethylation (VI) greater than dimethylation (VII) and suggested that the increased steric hindrance of methyl at high temperature was the deciding factor.

(1) Supported in part by grants A-4044 and CY-4550, U. S. Public Health Service.

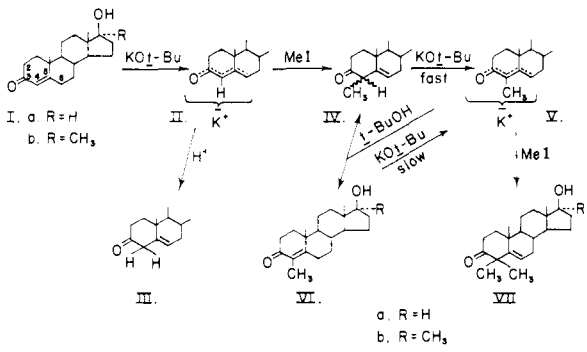
(2) *Cf.* J. M. Conia and M. A. Le Craz, *Bull. Soc. Chim.*, 1327 (1960).

(3) (a) R. B. Woodward, A. A. Patchett, D. H. R. Barton, D. A. J. Ives and R. B. Kelly, *J. Am. Chem. Soc.*, **76**, 2852 (1954); (b) G. Cooley, B. Ellis and V. Petrow, *J. Chem. Soc.*, 2998 (1955); (c) H. J. Ringold and G. Rosenkranz, *J. Org. Chem.*, **22**, 602 (1957); (d) F. Sondheimer and Y. Mazur, *J. Am. Chem. Soc.*, **79**, 2906 (1957).

(4) M. Yanagita, M. Hirakura and F. Seki, *J. Org. Chem.*, **23**, 841 (1958).

(5) N. W. Atwater, *J. Am. Chem. Soc.*, **82**, 2847 (1960).

Yanagita and co-workers,[4] who found that the room temperature alkylation of 10-methyl-$\Delta^4$-octalin-3-one with methyl iodide–potassium *t*-butoxide–*t*-butyl alcohol readily gave the 4,4-dimethyl-$\Delta^5$-3-one although 4,10-dimethyl-$\Delta^4$-octalin-3-one was unreactive under these conditions, were unable to rationalize their results.




Our recent finding[6] that ring A-unsubstituted $\Delta^4$-3-keto steroids (I) may be almost completely deconjugated to the $\Delta^5$-3-ones (III) by the acetic acid protonation of the conjugate anion (II) which had been formed with potassium *t*-butoxide in *t*-butyl alcohol allowed a study of this alkylation reaction in detail and an explanation of the apparent anomalies. When 4-methyltestosterone (VIa) and 4,17-dimethyltestosterone (VIb) were submitted to deconjugation treatment (10 equiv. of potassium *t*-butoxide, 1.5 hours and acetic acid protonation), only negligible deconjugation was observed by ultraviolet and infrared spectroscopy. Comparative alkylations of 17$\alpha$-methyltestosterone (Ib) and of 4,17$\alpha$-dimethyltestosterone (VIb) (10 equiv. of potassium *t*-butoxide, 10 minute stirring, then addition of 5 equiv. of methyl iodide with an additional 10 minute reaction time at room temperature) were carried out. Although Ib led to 74% of 4,4,17-trimethyl compound[3c] (VIIb), VIb gave only 6% of VIIb and was recovered unchanged in 84% yield. However, when VIb was allowed to react with 10 equiv. of potassium *t*-butoxide for 65 hours prior to the addition of methyl iodide and then allowed 10 minutes reaction time with the alkyl halide, 67% of 4,4,17-trimethyl compound (VIIb) was isolated. The treatment of testosterone (Ia) and 4-methyltestosterone (VIa) in a similar fashion gave reaction patterns resembling the 17$\alpha$-methyl cases.

Thus the slow rate of alkylation of the 4-methyl-$\Delta^4$-3-ketones (VI) is due to a slow rate of formation of the conjugate anion (V) and the 4-methyl-$\Delta^4$-3-ketone cannot be the major intermediate in the formation of the 4,4-dimethyl compound from I. Just as irreversible protonation of the conjugate anion (II) leads to the deconjugated ketone, methylation of this anion must yield the 4-methyl-$\Delta^5$-3-ketone (III) as the primary reaction product. Since the 4-proton of III will be considerably more acidic than the 6-proton of either I or of VI, formation of the 4-methyl conjugate anion (V) will proceed rapidly. At this point two competing reactions which will affect product determination can occur: (1) alkylation at C-4, (2) formation of the 4-methyl-$\Delta^4$-3-ketone by protonation at C-6.[7] At room temperature and in the presence of excess methyl iodide, (1) is faster than (2), which leads to the 4,4-dimethyl compound (VII) as the major product. Under the Atwater[5] conditions equilibration to the 4-methyl-$\Delta^4$-3-ketone (VI) becomes more rapid than alkylation. Once VI has been formed, conjugate anion production is very slow compared to the unmethylated compound (I) and the major product becomes the 4-monomethylated derivative (VI).

Protonation of the conjugate anion formed after 4,17-dimethyltestosterone had been in 65 hour contact with 10 equiv. of base revealed only about 20% deconjugation although from the corresponding alkylation experiment a minimum of 65% of conjugate anion must have been present. A decision between preferential protonation of V at C-6, or double bond shift post-protonation at C-4, readily was provided by deuteration of the conjugate anion with deuterioacetic acid whence 60% deconjugation of the 4-methyl compound occurred demonstrating that protonation did proceed primarily at C-4 and the rapid double bond rearrangement could be retarded by deuterium substitution at C-4.[8]

Our results suggested that monomethylation would be favored over dimethylation simply by substituting methyl chloride for methyl iodide which would slow the rate of the second alkylation while the rate of conjugation to the relatively unreactive 4-methyl-$\Delta^4$-3-one (VI) would not be affected. A mixture of $\Delta^4$-cholesten-3-one (400 mg.) and potassium *t*-butoxide (3 equiv.) in 15 ml. of anhydrous *t*-butyl alcohol was stirred for 5 hours under nitrogen to pre-form the conjugate anion and the solution then saturated with methyl chloride and allowed to stand for 5 hours at room temperature.[9] Re-saturation and an additional 16 hours reaction time gave 40% 4-methyl-$\Delta^4$-cholesten-3-one,[3d] 12% 4,4-dimethyl-$\Delta^5$ cholesten-3-one[3a] and 35% starting material. 17$\alpha$-Methyltestosterone, under similar reaction conditions, gave 30% VIb and 11% VIIb. In contrast, methyl iodide under identical anion preformation conditions favored 4,4-dimethylation over 4-monomethylation by ratios of 6:1 and 13:1 with the two substrates cited. This altered ratio demonstrates the validity of the proposed reaction path.

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RECEIVED JUNE 28, 1962

(6) H. J. Ringold and S. K. Malhotra, *Tetrahedron Letters*, 669 (1962).

(7) It is recognized that protonation at C-4 also occurs but this product will be readily re-converted to V by base.

(8) An alternate measure of the rate of anion formation in *t*-butyl alcohol has been provided by quenching of the reaction mixture (10 equiv. base, 10 minutes reaction) with excess acetic anhydride which led to complete $\Delta^{3,5}$-enol acetate formation in the case of testosterone while 4-methyltestosterone (VIa) was recovered unchanged. Extension of the reaction time of VIa with base to 110 hours, then anhydride addition, gave complete enol acetate formation.

(9) When methyl chloride was introduced into the solution immediately after the addition of *t*-butoxide, C-alkylation was too slow to compete with the reaction of the alkoxide and the alkyl halide.