be used as a measure of the resonance effect of a substituent. If this conclusion is valid, the order of groups in Table II should parallel their conjugative ability. It is immediately obvious that the order of groups in Table II is quite different from the order in Table I. In particular, the carboethoxy group is higher than five other groups in Table II, but lower than the same five groups in Table I. It is also seen that the spectrum of tetraphenylsilane indicates that little or no resonance exists between the silicon atom and the benzene ring. An investigation[4c] of substituent constants, on the other hand, led to the opposite conclusion.

TABLE II

THE EFFECT OF SUBSTITUENTS ON THE PRIMARY (203.5 mμ) BAND OF BENZENE

C_6H_5X X =	$\lambda_{max} - 203.5$ mμ
NO_2	65.0[a]
CHO	46.0[a]
$COCH_3$	42.0[a]
$COOC_2H_5$	27.0[b]
COOH	26.5,[a] 26.5[c]
CN	20.5[a]
$S(CH_3)_2^+$	16.5[d]
SO_2CH_3	13.5[e]
$Si(C_6H_5)_3$	0[f]

[a] Taken from ref. 6. [b] The ultraviolet absorption spectrum of ethyl benzoate was determined in 2% methanol according to the procedure previously described by Jaffé and Freedman, *J. Am. Chem. Soc.*, **74**, 1069 (1952); maxima at 230.5 mμ and 273 mμ (ε 11,100 and 890 respectively). [c] The spectrum of benzoic acid was determined in 0.1 *N* hydrochloric acid; maxima at 230 mμ and 273 mμ (ε 11,600 and 990 respectively). [d] Taken from ref. 4b; water was used as the solvent. [e] Taken from Fehnel and Carmack, *J. Am. Chem. Soc.*, 71, 231 (1949); absolute ethanol was used as the solvent.

[f] The spectrum of tetraphenylsilane was determined in 95% ethanol; maxima at 203.5 mu, 254 mu, 260 mu, and 264.5 mu (ε 48,000, 1480, 1590, and 1450 respectively). The spectrum of this compound was previously determined by Milazzo, *Gazz. chim. ital.*, **71**, 73 (1941); *Chem. Abstr.*, **37**, 1928 (1943).

It should be emphasized, we believe, that the values of substituent constants are subject to appreciable errors.[7] Since the differences between σ^* and σ are, of course, liable to even larger errors, it seems unwise to rely on the magnitude of these differences as a measure of the conjugative ability of substituent groups.

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(7) *Cf.* Table 8 of ref. 2.

Reactions of Organometallic Compounds with Pyridazine[1]

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Received March 12, 1956

We have observed that pyridazine can be alkylated (or arylated) by means of organometallic compounds, and that such reactions may be used for the preparation of either 3- or 4-substituted pyridazines. Data on four typical reactions are summarized in Table I.

TABLE I

REACTIONS OF ORGANOMETALLIC COMPOUNDS WITH PYRIDAZINE

Reaction	Organometallic Compound	Solvent	Position of Substitution in Isolated Product	Percentage Yield of Substituted Pyridazine
1	C_6H_5MgBr	Ether	4	7.3
2	C_6H_5Li	Ether	3	17
3	C_4H_9MgBr	Ether-tetrahydrofuran	4	23
4	C_4H_9Li	Ether	3	51

4-Substituted pyridazines were isolated from the Grignard reactions, and 3-substituted derivatives from the lithium reactions. The solvent is also a factor in the orientation, for a mixture of 3- and 4-butylpyridazine was obtained from a reaction of butyllithium with pyridazine in a mixed ether-tetrahydrofuran solvent.

The phenylpyridazines were characterized by their melting points and by derivatives; and the butylpyridazines, which were liquids, by oxidation to the known 3- and 4-pyridazinecarboxylic acids. Since the yields of the acids from these oxidations were of the order of 32–41%,[2] the possibility cannot be excluded that the products from reactions 3 and 4 were contaminated by some of the isomeric compound. The substance labeled 4-butylpyridazine seems to be essentially pure, however, for it was converted in 93% yield to a *picrate*, which melted at 107.5–108.5°. 3-Butylpyridazine did not form a crystalline picrate. Substituted pyridazines were

(1) Financial support for this work came from the Research Corporation.

(2) Variable yields have been reported for the pyridazinecarboxylic acids produced from oxidation reactions. Thus, (a) Poppenberg, *Ber.*, **34**, 3267 (1901), was unable to isolate any carboxylic acid from an oxidation of 3-methylpyridazine. On the other hand, (b) Leanza, Becker, and Rogers, *J. Am. Chem. Soc.*, **75**, 4086 (1953), obtained an 81% yield of 3-pyridazinecarboxylic acid from 3-hydroxymethylpyridazine, and (c) Gabriel and Colman, *Ber.*, **32**, 407 (1899), a 28% yield of the same acid from 3-*p*-hydroxyphenylpyridazine.

isolated directly from reactions 1–3; however, the product from 4 was a dihydropyridazine which was subsequently oxidized to the aromatic compound by potassium permanganate in acetone.

These reactions are similar to those shown by pyridine and quinoline. Although phenylmagnesium bromide in ether merely forms a complex with pyridine at room temperature,[3] it reacts slowly with quinoline to give 2-phenylquinoline (7.4% from a two day reaction.)[4] At 150° pyridine also yields 2-phenylpyridine.[3] Organolithium compounds react more readily than do the Grignard reagents; indeed, these reactions provide a convenient synthetic route to alkyl and aryl pyridines and quinolines.[5] In all these cases, however, the isolable products were exclusively, or very extensively the 2-isomers. The only reaction for which attack at the 4-position predominated seems to be that of benzylmagnesium chloride with quinoline (the ratio of *para* to *ortho* substitution was about 4:1).[6] Dihydro derivatives, which were the initial products from some of the pyridine and quinoline reactions, were generally oxidized by air[5d] or nitrobenzene.[4,5c]

EXPERIMENTAL

4-Phenylpyridazine. An ether solution (150 ml.) of phenylmagnesium bromide (0.12 mole) was added over a period of 15 minutes to a stirred solution of 8.00 g. (0.1 mole) of pyridazine[7] in 100 ml. of ether at a temperature of −20°. During the addition a yellow-brown solid separated. While stirring was continued, the mixture was allowed to warm to room temperature (45 minutes); then was refluxed for two hours and decomposed with aqueous ammonium chloride. The aqueous layer was extracted with 500 g. of chloroform in portions; the extracts then were combined with the ether layer, dried, and distilled at 3 mm. to give three fractions: (a) b.p. 59–60°; weight, 3.0 g.; (b) b.p. 69–99°; 0.70 g.; (c) b.p. 99–159°; 2.74 g. When (c) was washed with ether, it yielded 1.28 g. of solid material, m.p. 68–78°. Recrystallization from hexane gave 0.90 g. of 4-phenylpyridazine, m.p. 82–83°. A second recrystallization raised the melting point to 83.5–84°. The melting point reported[8] for 4-phenylpyridazine is 86–86.5°.

Anal.[9] Calc'd for $C_{10}H_8N_2$: C, 76.90; H, 5.16. Found: C, 76.58; H, 5.12.

When the mother liquors from these crystallizations were treated with picric acid, there was obtained 0.68 g. of a picrate, m.p. 145.5–148°; m.p. after recrystallization, 149–150°. This did not depress the melting point of a sample of 4-phenylpyridazine picrate (m.p. 149–150°) prepared directly from the pyridazine and picric acid.

Anal. Calc'd for $C_{16}H_{11}N_5O_7$: C, 49.87; H, 2.88. Found: C, 49.25; H, 3.15.

The total yield of 4-phenylpyridazine was 7.3% (0.90 g. obtained directly and 0.25 g. obtained in the form of the picrate).

Fraction (a) was pyridazine (39% recovery). Its picrate melted at 171–172° and did not depress the melting point of authentic pyridazine picrate.

A similar reaction was carried out in which the Grignard reagent was added to the ether solution at room temperature, followed by two hours of reflux. In this case the organic liquid which remained after removal of the ether and chloroform (used in the extraction as before) was boiled in 15 ml. of nitrobenzene in order to oxidize any dihydro material which might have been present. From this reaction there was isolated 0.98 g. (6.3%) of 4-phenylpyridazine.

3-Phenylpyridazine. A filtered solution (325 ml.) of phenyllithium (0.115 mole) in ether was added over a 30-minute period to 8.00 g. (0.10 mole) of pyridazine in 100 ml. of ether at −5°. At the end of the addition an additional 200 ml. of ether was added in order to dissolve most of the reddish-yellow precipitate which had separated. After 30 minutes, the cooling bath was removed and the solution was stirred for an additional three hours. Hydrolysis, extraction, and distillation as in the previous case yielded 6.78 g. of product which boiled from 122–150° (1 mm.). On crystallization from hexane and from water, several crystalline fractions were obtained, all of which melted in the range between 98 and 100.5° and the purest portion at 99.5–100.5°. Altogether, the yield of 3-phenylpyridazine was 2.65 g. (17%). The picrate and methiodide derivatives melted at 126–127° and 176.5–178°, respectively. Reported melting points[8] for 3-phenylpyridazine and the picrate and methiodide derivatives are, respectively: 102–103°; 127°; and 179°.

Another reaction was carried out similarly except that the reaction products were heated with nitrobenzene. In this case the yield of 3-phenylpyridazine (samples melting within the range of 95–100.5°) was 19%.

4-n-Butylpyridazine. An ether solution (300 ml.) of butylmagnesium bromide (0.116 mole) was added over a period of 30 minutes to 8.00 g. (0.100 mole) of pyridazine in 500 ml. of tetrahydrofuran at −18° to −12°. The solution was stirred for 30 minutes, and allowed to warm to room temperature overnight. After removal of most of the solvent, benzene was added and distillation was continued until little tetrahydrofuran remained. Water then was added, and the aqueous layer was saturated with sodium carbonate and extracted with ether. Distillation yielded 3.10 g. (23%) of butylpyridazine; b.p. 97–102° (10 mm.); n_D^{26} 1.4978. When treated with an alcoholic solution of picric acid, a sample of this was converted in 93% yield to the picrate, m.p. 107.5–108.5°. After recrystallization it melted at 110.5–111°.

Anal. Calc'd for $C_{14}H_{15}N_5O_7$: N, 19.17. Found: N, 19.65.

Oxidation of 2.0 g. of the butylpyridazine at 60–70° for four hours with 8.73 g. of potassium permanganate in 500 ml. of water which contained 0.75 g. of potassium hydroxide gave 0.75 g. of a solid acid, m.p. 234–240° (dec.). This corresponds to a 41% yield of 4-pyridazinecarboxylic acid. On recrystallization from water, 0.70 g. of product was obtained which melted with decomposition at 240–242°.

(3) Bergstrom and McAllister, *J. Am. Chem. Soc.*, **52**, 2845 (1930). These authors claimed to have obtained a 66% yield of 2-ethylpyridine from ethylmagnesium bromide and pyridine at 150°; however, Gaetz-Luthy, *J. Am. Chem. Soc.*, **71**, 2254 (1949), repeated this work and was unable to find any ethylpyridine in the reaction products.

(4) Gilman and Gainer, *J. Am. Chem. Soc.*, **71**, 2327 (1949). With a mixed ether-dioxane solvent and other conditions the same, the yield of 2-phenylquinoline was 44%.

(5) (a) Ziegler and Zeiser, *Ann.*, **485**, 174 (1931); *Ber.*, **63**, 1847 (1930); (b) Evans and Allen, *Org. Syn.*, **Coll. Vol. 2**, 517 (1943); (c) Gilman and Gainer, *J. Am. Chem. Soc.*, **69**, 877 (1947); (d) Gilman and Broadbent, *J. Am. Chem. Soc.*, **70**, 2809 (1948); (e) Gilman and Spatz, *J. Am. Chem. Soc.*, **63**, 1553 (1941).

(6) Benkeser and Holton, *J. Am. Chem. Soc.*, **73**, 5861 (1951).

(7) For preparation of the pyridazine see Letsinger and Lasco, *J. Org. Chem.*, **21**, 764 (1956).

(8) Grignard, DuPont, and Locquin, *Traite de Chimie Organique*, Vol. **XX**, p. 980–981.

(9) Analyses were carried out by Miss H. Beck and Miss C. White.

4-Pyridazinecarboxylic acid is reported to melt at 239–240° with decomposition.[2b]

3-Butylpyridazine. To a solution of 8.0 g. of pyridazine in 500 ml. of ether was added (30 min.) 246 ml. of 0.477 *M* *n*-butyllithium (0.116 mole). The temperature was maintained at −15°. After 30 minutes stirring, the mixture was allowed to warm to room temperature overnight, then was hydrolyzed with water. Extraction of the aqueous layer with ether and distillation of the organic portion yielded 9.15 g. (66.3% calculated as butyldihydropyridazine) of product; b.p. 61.5–66.5° (0.5 mm.); $n_D^{25.2}$ 1.4940. The infrared spectra, in contrast to that for the 4-butylpyridazine obtained by the previously described reaction, showed a strong absorption peak at 3.02 microns, no strong peak at 6.30 microns, and the 7–12 region was unlike that of the pyridazines. These data indicate that the product was the dihydro compound, as confirmed by the analysis.

Anal. Calc'd for $C_8H_{14}N_2$: C, 69.52; H, 10.21. Found: C, 69.98; H, 10.17.

The dihydro compound (3 g.) was oxidized with 2.29 g. of potassium permanganate in 450 ml. of acetone at a temperature which did not exceed 15°. Distillation of the organic products gave 0.25 g. b.p. 77–79.5° (1 mm.) and 2.08 g., b.p. 79.5–82° (1 mm.); $n^{25.2}$ 1.4950 [total yield b.p. 77–82° (1 mm.), 2.33 g. (77%)].

Anal. Calc'd for $C_8H_{12}N_2$: C, 70.55; H, 8.89. Found: C, 71.15; H, 8.91.

No crystalline picrate could be obtained from this material. The infrared spectra was characteristic of a pyridazine. Absorption at 3.12 microns was weak and there were good peaks at 6.30 and 6.98 microns. Furthermore, the 7–12 region resembled that of 3-methylpyridazine.

A 2.00-g. sample of the butylpyridazine was oxidized under the conditions used for the 4-butylpyridazine. In this case, a 32% yield of 3-pyridazinecarboxylic acid, m.p. 199–200° (dec.), was obtained as the only isolable acid. On recrystallization from water, 0.3 g. of acid was isolated which melted with decomposition at 203.5–204.5°; the melting point reported for 3-pyridazinecarboxylic acid is 201° (with decomposition).[2c]

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Degradation of Cortisol-C^{14} and Corticosterone-C^{14} Biosynthesized from Acetate-1-C^{14} [1,2]

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Received March 12, 1956

Acetate is known to be a precursor of cholesterol and of various steroid hormones. The arrangement of the majority of carbons incorporated into the cholesterol molecule from acetate is known with certainty.[3–6] On the basis of these findings together with the observation that squalene may be converted to cholesterol, Woodward and Bloch[5] postulated the arrangement of methyl and carboxyl carbons derived from acetate in the cholesterol molecule. This communication is concerned with the arrangement of some of the methyl and carboxyl carbons in cortisol and corticosterone derived by biosynthesis from acetate-1-C^{14}.

EXPERIMENTAL

Calf adrenal glands were perfused with a glucose-fortified, physiologically balanced and buffered salt solution containing acetate-1-C^{14} and gased with 95% O_2/5% CO_2 as described by Rosenfeld.[7] Two perfusions were done, one using 3 mc and the other 5 mc of acetate-1-C^{14}. In each case the glands were perfused for two hours at 37.5° in a multicycle system with one liter of fluid.

The perfusates were extracted three times with two volumes of isopropyl acetate. The extracts were washed with a saturated solution of sodium bicarbonate and water until neutral. The isopropyl acetate extract was dried over sodium sulfate and evaporated *in vacuo* to yield a neutral residue containing 2.62×10^6 counts per minute (c/m). The sodium bicarbonate solution was acidified with hydrochloric acid, extracted with isopropyl acetate, washed with water, dried over sodium sulfate, and evaporated *in vacuo* to yield an acidic residue containing 0.5×10^6 c/m.

Carrier cortisol (5 mg.) and corticosterone (5 mg.) were added to the neutral residue which then was chromatographed on paper in the toluene-propylene glycol system (TPG) for 78 hours. The run off was rechromatographed for 16 hours in the TPG system. Cortisol and corticosterone were detected by radioautography, scanning under the ultraviolet lamp, and the blue tetrazolium reaction. After each compound was rechromatographed twice on paper, single spots were detected. The eluted material then was chromatographed on a partition column using ethanol-water (1:3) as the stationary phase on a Celite support and benzene as the mobile phase. The hormones, which came off the columns as single peaks, were further diluted with nonradioactive steroids and were recrystallized ten times to a constant specific activity. The C^{14}-labeled hormones, of constant specific activity, were combusted and were counted as carbon dioxide. A total of 192 mg. of cortisol-C^{14} of 0.525×10^6 disintegrations per minute per millimole (d/m/mM) and 190 mg. of corticosterone-C^{14} of 0.635×10^6 d/m/mM were obtained.

The ketolic side chain of cortisol-C^{14} was cleaved with sodium bismuthate[8,9] and C-21 was isolated as the formadone and C-20 as barium carbonate. Corticosterone was similarly reacted with sodium bismuthate and C-21 again was isolated as the formadone. The products were counted as carbon dioxide. No C^{14} could be detected in C-21 derived from either cortisol or corticosterone. The sensitivity of the counting procedure would permit the finding of 3880 d/m/mM for the cortisol carbon and 5250 d/m/mM for the corticosterone

(1) These studies were aided in part by Contract No. AT(30-1)-918, U. S. Atomic Energy Commission; and by Contract No. N6ori-197 between the Office of Naval Research, Department of the Navy, and the Worcester Foundation for Experimental Biology.

(2) The opinions or assertions contained herein are the private ones of the author and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.

(3) Wüersch, Huang, and Bloch, *J. Biol. Chem.*, **195**, 439 (1952).

(4) Cornforth, Hunter, and Popjak, *Biochem. J.*, **54**, 597 (1953).

(5) Woodward and Bloch, *J. Am. Chem. Soc.*, **75**, 2023 (1953).

(6) Dauben and Takemura, *J. Am. Chem. Soc.*, **75**, 6302 (1953).

(7) Rosenfeld, *Endocrinology*, **56**, 649 (1955).

(8) Rigby, *J. Chem. Soc.*, 1907 (1950).

(9) Appleby, Gibson, Norymberski, and Stubbs, *Biochem. J.*, **60**, 453 (1955).