achieved by these methods. The optimum eluting solvent was 1% (v/v) EtOH in $CHCl_3$ for hydrocortisone and 3% (v/v) EtOH in $CHCl_3$ for dexamethasone.

Table I summarizes the apparent molar absorption coefficients and the absorption maxima in some Δ^4- and $\Delta^{1,4}$-3-ketosteroids. Concerning $\Delta^{1,4}$-3-ketosteroids, the absorbancy obtained with INH.2HCl reagent was larger than that with INH reagent. The results of the analyses of dexamethasone and hydrocortisone in some formulations are shown in Table II. The results are quite satisfactory. The combination of the column chromatography-colorimetry procedures could be generally applicable in various types of pharmaceutical preparations.

TABLE II. Results of Ketosteroids in Some Formulations determined by the Present Method

Steroids (formulation)	Nominal (%)	Found[a] (%)	Standard deviation (%)
Dexamethasone (ointment)	0.05	0.051	±1.2
Dexamethasone (cream)[b]	0.025	0.0252	±0.9
Dexamethasone (lotion)	0.05	0.052	±1.1
Hydrocortisone (suppository)[b]	0.05	0.050	±1.0

a) mean value of five determinations
b) Separation of steroid was carried out by using the column chromato graphy. Hydrocortisone was treated with the method indicated in Fig. 4 (A).

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Benzodiazepines. VI.[1] A Rearrangement of 2-Aminoacetanilides to Anilinoacetamides

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In addition to the expected 7-nitrobenzodiazepin-2-one (**2**), we have isolated a small amount of 2-(2-benzoyl-4-nitroanilino)-N-methylacetanilide (**5**) as a by-product from the chromic acid oxidation of the 2-aminomethyl-5-nitroindole (**1**).[1] It has been postulated that the conversion of **1** to **5** involves, initially oxidative opening of **1** to the intermediate **3**, which then rapidly rearranges to **5**. Rearrangement is thought to proceed through the cyclic transition state (**4**) by intramolecular nucleophilic attack of the amino group on the

1) Part V: S. Inaba, K. Ishizumi, K. Mori and H. Yamamoto, *Chem. Pharm. Bull.* (Tokyo), **19**, 722 (1971).
2) Location: *2-1, Takatsukasa-4-chome, Takarazuka-shi, Hyogo.*

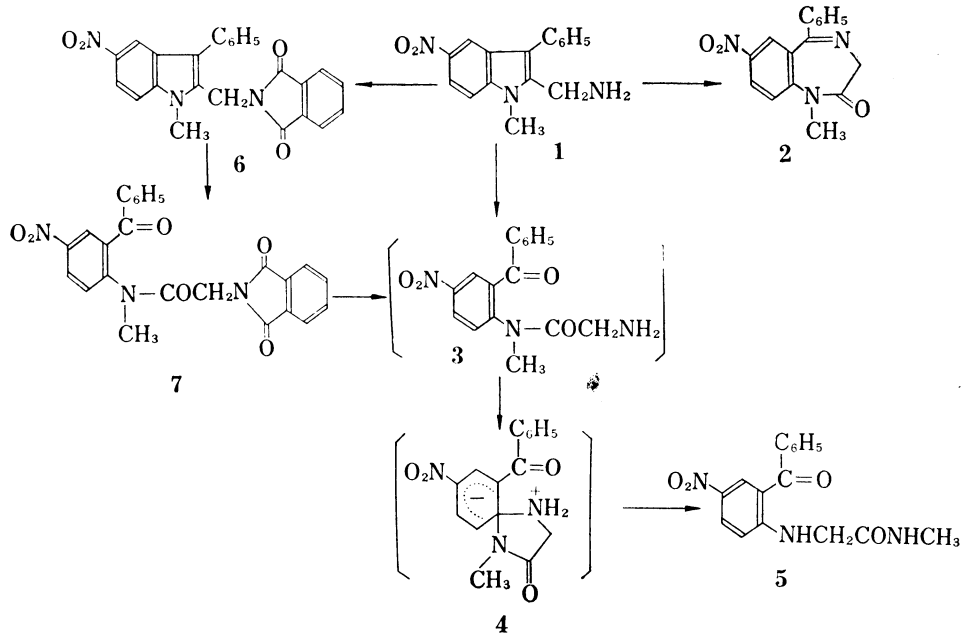

Chart 1

aromatic carbon *para* to the nitro group.[3] In this note we wish to report the further investigations of this rearrangement.

2′-Benzoyl-N-methyl-4′-nitro-2-phthalimidoacetanilide (**7**) was prepared from **1** by fusion with phthalic anhydride to give the corresponding 2-phthalimidomethylindole (**6**), which was subsequently oxidized to **7**. Phthalimido cleavage of **7** by aqueous hydrazine hydrate resulted in nearly quantitative conversion to **5**.

From this observation, it is clear that in the formation of **5** from **1**, **3** is an intermediate which rearranges so rapidly to **5** that it can not be isolated. Furthermore, more recently Gilman and Sternbach[4] reported that the rearranged acetanilide was the only product obtained by the reaction of 2′-benzoyl-2-bromo-N-*t*-butyl-4′-nitro-acetanilide with methanolic ammonia.

The mechanism for the formation of **2** from **1** is difficult to explain. However, the facts that the 2-aminoacetanilides such as **3** afford only the rearranged products make it appear that the reaction proceeds by a pathway which does not involve **3**.

On the other hand, the same hydrazine treatment of the desmethyl derivative (**8**) led only to the formation of the unrearranged 2-aminoacetanilide (**9**).[7] It was then found that

3) A similar mechanism through five-membered transition state has been reported by R. Dohmori[5] for the S→C aryl migration, and by others[6] for a variety of intramolecular S→N, O→O′, O→N and N→N′ aryl migrations.

4) N.W. Gilman and L.H. Sternbach, *J. Heterocycl. Chem.*, **8**, 297 (1971).

5) R. Dohmori, *Chem. Pharm. Bull.* (Tokyo), **12**, 601 (1964).

6) *a*) H.P. Burchfield, *Nature*, **181**, 49 (1958); *b*) M. Harfenist and E. Thom, *J. Chem. Soc.* (*D*), 730 (1969); *c*) K.G. Kleb, *Angew. Chem.*, **80**, 284 (1968); *d*) N.W. Gilman, P. Levitan and L.H. Sternbach, *Tetrahedron Letters*, 4121 (1970).

7) TLC analysis indicated that the reaction was accompanied by a trace of the cyclized benzodiazepin-2-one (**10**). It was reported[8] that **8** in methanol was refluxed with 18% aq. hydrazine to form **10** in 83% yield.

8) H. Rochnert, F. Bahr and E. Carstens, German (East) Patent 57126 (Aug. 5, 1967) [*C. A.*, **69**, 36192c (1968)].

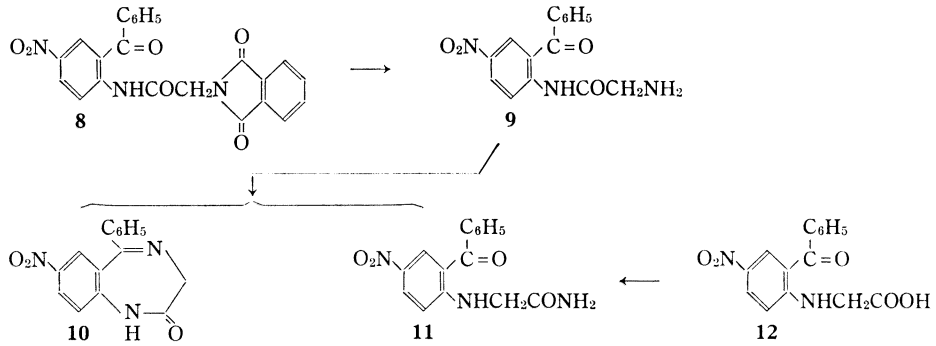

Chart 2

2-Amino-2′,4′-dinitroacetanilide (**14**), in which benzoyl group of **9** is replaced by nito group, was prepared from 2-bromo-2′-4′-dinitroacetanilide (**13**) by treatment with ammonia. As expected, the anilide **14**, dissolved in dimethyl sulfoxide, afforded only the rearranged product (**15**).

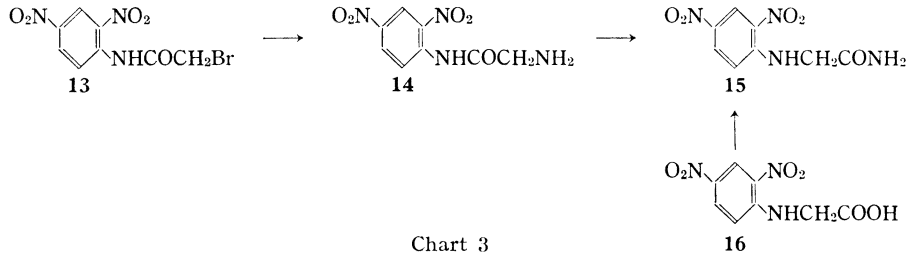

Chart 3

warming of **9** in dimethyl sulfoxide gave the cyclized benzodiazepin-2-one (**10**) and a trace of the rearranged anilinoacetamide (**11**).

Anilinoacetamides **11** and **15** were identified with authentic samples prepared by treatment of the mixed anhydride of N-phenylglycines **12**[1] and **16**[9] with ammonia.

Thus, this intramolecular N→N′ aryl migration of the Smiles rearrangement type appears to be general for 2-aminoacetanilides bearing electron attracting group in the *ortho* or *para* position.

Experimental

All melting points were determined in open capillary tubes and are uncorrected. Infrared (IR) spectra were measured on a Hitachi Model EPI-G3 spectrophotometer, ultraviolet (UV) spectra on a Shimadzu SV-50-AL spectrophotometer and nuclear magnetic resonance (NMR) spectra on a Varian A-60-D instrument using tetramethylsilane as an internal standard. Thin-layer chromatography (TLC) was done on 0.25 mm layers of Merck silica gel GF_{254}. All solvents were evaporated on a rotary evaporator under water aspirator pressure.

1-Methyl-5-nitro-3-phenyl-2-phthalimidomethylindole (6)——A mixture of 3.15 g of 2-aminomethyl-1-methyl-5-nitro-3-phenylindole (**1**) and 2.0 g of phthalic anhydride was heated to 260° for 15 min. The resulting mixture was cooled and washed with tetrahydrofuran to give 4.14 g (90%) of **6**, mp 284—285°. Recrystallization from tetrahydrofuran afforded an analytical sample as yellow needles, mp 284.5—285°. IR ν_{max}^{Nujol} cm^{-1}: 1773, 1706 and 1613. *Anal.* Calcd. for $C_{24}H_{17}O_4N_3$: C, 70.06; H, 4.17; N, 10.21. Found: C, 70.29; H, 4.20; N, 10.00.

2′-Benzoyl-N-methyl-4′-nitro-2-phthalimidoacetanilide (7)——To a stirred suspension of 3.9 g of **6** in 35 ml of acetic acid was added a solution of 2.92 g of chromium trioxide in 3 ml of water. The mixture was stirred at room temperature for 8 hr, and then diluted with water. The resulting precipitate was col-

(9 Prepared according to the method of K.R. Rao and H.A. Sober [*J. Am. Chem. Soc.*, **76**, 1328 (1954)].

lected by filtration and recrystallized from tetrahydrofuran to give 2.33 g (56%) of **7** as prisms, mp 220—221.5°. IR ν_{max}^{Nujol} cm^{-1}: 1775, 1719, 1675, 1662, and 1612. NMR (DMSO-d_6) δ: 2.98 and 3.38 (3H, CH_3), 4.20 and 4.30 (2H, CH_2) and 7.7—8.7 (12H, m, aromatic). *Anal.* Calcd. for $C_{24}H_{17}O_6N_3$: C, 65.01; H, 3.86; N, 9.47. Found: C, 65.28; H, 3.99; N, 9.42.

2-(2-Benzoyl-4-nitroanilino)-N-methylacetamide (5)——To a suspension of 1.0 g of **7** in a mixture of 10 ml of chloroform and 10 ml of ethanol was added 1.0 g of 50% aq. hydrazine hydrate. The mixture was stirred at room temperature for 22 hr. The solvent was evaporated and the residual solid was washed with water, dried and chromatographed on 25 g of silica gel. After removal of fast moving impurities by elution with chloroform, 0.62 g (88%) of **5** was eluted using a mixture of ethyl acetate and ethanol (9: 1). Recrystallization from ethanol gave pure **5** as yellow needles, mp 242—243°, identical with an authentic sample[1] by mixture melting point, IR and TLC.

5-Nitro-3-phenyl-2-phthalimidomethylindole (17)——A mixture of 5.0 g of 2-aminomethyl-5-nitro-3-phenylindole (**18**)[10] and 2.8 g of phthalic anhydride was heated to 180° for 5 min. Work-up as described above for the preparation of **6** and recrystallization from tetrahydrofuran gave 4.05 g (54%) of pure **17** as prism, mp 237—238°. IR ν_{max}^{Nujol} cm^{-1}: 3331, 1770, 1710, 1624, and 1605. *Anal.* Calcd. for $C_{23}H_{15}O_4N_3$: C, 69.51; H, 3.81; N, 10.58. Found: C, 69.65; H, 3.74; N, 10.56.

2′-Benzoyl-4′-nitro-2-phthalimidoacetanilide (8)——This compound was prepared from **17** by a procedure similar to that described above for the preparation of **7**. Recrystallization of the crude solid from methanol gave 2.04 g (63% from 3.0 g of **17**) of pure **8**, mp 198.5—199° (lit.[8] mp 198—204°). IR ν_{max}^{Nujol} cm^{-1}: 3225, 3098, 3053, 1772, 1733, 1713, 1641, 1615, and 1600. NMR (DMSO-d_6) δ: 4.35 (2H, s, CH_2), 7.5—8.6 (12H, m, aromatic) and 10.90 (1H, s, D_2O exchangeable, NH). *Anal.* Calcd. for $C_{23}H_{15}O_6N_3$: C, 64.33; H, 3.52; N, 9.79. Found: C, 64.50; H, 3.44; N, 9.86.

2-Amino-2′-benzoyl-4′-nitroacetanilide (9)——The above anilide **8** was treated as described for the hydrazine cleavage of **7**. After washing with ether, 0.48 g (69% from 1.0 g of **8**) of crude **9** was obtained. The crude **9** was dissolved in 20 ml of chloroform and filtered from the insoluble material. To the filtrate was added 50 ml of ether and the solution was cooled. The precipitate formed was filtered and dried to give 0.25 g (36%) of pure **9** as slightly yellow needles, mp 167° (decomp.) [lit.[11] mp 166—167° (decomp.)]. IR ν_{max}^{Nujol} cm^{-1}: 3400, 3325, 3170, 1702, 1643, and 1611. *Anal.* Calcd. for $C_{15}H_{13}O_4N_3$: C, 60.19; H, 4.38; N, 14.04. Found: C, 59.81; H, 4.07; N, 13.76.

Treatment of 9 with Dimethyl Sulfoxide——A suspension of 1.0 g of **9** in 5 ml of dimethyl sulfoxide was stirred at room temperature for 1 hr. TLC analysis (ethyl acetate) indicated that most of **9** remained unchanged. The reaction mixture was heated to 60°. The reaction was shown by TLC analysis to be complete in 2 hr. Approximately half the dimethyl sulfoxide was evaporated and to the residue was added 10 ml of ethanol, followed by the addition of 100 ml of petroleum ether. The precipitate formed was collected by filtration and dried to give 0.70 g of **10**, mp 212—217°. The mother liquor was evaporated and the residue was chromatographed on 30 g of silica gel. Elution with ethyl acetate gave first an additional 0.18 g of **10** and then 3 mg of **11**, mp 247—250° dec, IR spectrum and TLC identical with those of an authentic sample obtained from **12** below. Recrystallization of combined crops of **10** from ethanol gave 0.54 g (57%) of pure **10**, mp 223.5—225°, identical in all respects with an authentic sample.[12]

2-(2-Benzoyl-4-nitroanilino)acetamide (11)——A solution of 0.10 g of ethyl chloroformate in 5 ml of tetrahydrofuran was added with stirring to an ice-cooled solution of 0.2 g of N-(2-benzoyl-4-nitrophenyl)-glycine (**12**)[1] and 0.10 g of triethylamine in 15 ml of tetrahydrofuran. After 30 min, the resulting mixed anhydride solution was added under cooling and stirring to 15 ml of 28% aq. ammonia. The mixture was stirred at room temperature for 2 hr and tetrahydrofurane was evaporated. The precipitate formed was washed with water and collected by filtration to yield 0.19 g (95%) of **11**, mp 238—242° (decomp.). Two recrystallizations from ethanol gave analytically pure **11** as yellow needles; mp 254—256° (decomp.). IR ν_{max}^{Nujol} cm^{-1}: 3420, 3300, 3180, 3074, 3050, 1677, 1642 and 1607. NMR (DMSO-d_6) δ: 4.08 (2H, d, CH_2), 6.78—8.30 (3H, ABX type pattern, J_{ortho}=10 Hz, J_{meta}=3 Hz, H-3′, 5′, and 6′), 7.3 and 7.6 (2H, D_2O exchangeable, $CONH_2$), 7.61 (5H, s, C_6H_5) and 9.45 (1H, t, D_2O exchangeable, NH). *Anal.* Calcd. for $C_{15}H_{13}O_4N_3$: C, 60.19; H, 4.38; N, 14.04. Found: C, 59.90; H, 4.31; N, 14.00.

2-Bromo-2′,4′-dinitroacetanilide (13)——A mixture of 18.3 g of 2,4-dinitroaniline and 30.3 g of bromoacetyl bromide in 75 ml of benzene was refluxed for 8 hr until hydrogen bromide was no longer evolved. After cooling, the precipitate was collected by filtration, washed with cold benzene to give 22.7 g (75%) of **13**, mp 100—102°. An analytical sample was obtained by two recrystallizations from ethanol: mp 101—

10) Prepared from 5-nitro-3-phenylindole-2-carbonitrile[1] by reduction with sodium borohydride-borontrifluoride etherate. It crystallized from ethanol, mp 205—207°, NMR ($CDCl_3$) δ: 1.41 (2H, s, D_2O exchangeable, NH_2), 4.01 (2H, s, CH_2) and 9.40 (1H, broad, D_2O exchangeable, indole NH). *Anal.* Calcd. for $C_{15}H_{13}O_2N_3$: C, 67.40; H, 4.90; N, 15.72. Found: C, 67.69; H, 4.81; N, 15.43.

11) L.H. Sternbach, R.I. Fryer, O. Keller, W. Metlesics, G. Sach and N. Steiger, *J. Med. Chem.*, **6**, 261 (1963).

12) Obtained commercially.

103°. *Anal.* Calcd. for $C_8H_6O_5N_3Br$: C, 31.60; H, 1.99; N, 13.82; Br, 26.28. Found: C, 31.45; H, 1.97; N, 13.98; Br, 26.65.

2-Amino-2′,4′-dinitroacetanilide (14)——Ammonia gas was bubbled into a stirred solution of 10 g of **13** in a mixture of 100 ml of ethyl acetate and 100 ml of methylene chloride over a period of 8 hr at 25°. The resulting solution was filtered and evaporated. The residue was recrystallized from ethanol to give 4.7 g (60%) of **14** as plates, mp 151.5—152°. IR ν_{max}^{Nujol} cm^{-1}: 3407, 3330, 3160, 3085, 1702, 1613, and 1598. UV $\lambda_{max}^{CH_3OH}$ $m\mu$ (ε): 260 (11,100) and 330 (12,200). *Anal.* Calcd. for $C_8H_8O_5N_4$: C, 40.00; H, 3.36; N, 23.33. Found: C, 40.21; H, 3.26; N, 23.22.

2-(2,4-Dinitroanilino)acetamide (15). A. From 14——A solution of 1.0 g of **14** in 5 ml of dimethyl sulfoxide was stirred at room temperature for 7.5 hr. The rearranged acetamide **15** began to separate as orange crystals about 50 min after the beginning of the reaction. TLC analysis (ethyl acetate) showed that the reaction was nearly complete in 6 hr. The precipitate formed was collected by filtration and washed with ethanol. The crude **15** was suspended in 30 ml of ethanol and refluxed. Cooling and filtration afforded 0.70 g (70%) of **15** as yellow needles, mp 224—225°. Recrystallization from ethanol gave analytically pure **15**, mp 224—225°. IR ν_{max}^{Nujol} cm^{-1}: 3460, 3333, 3165, 3100, 3080, 1702, and 1614. UV $\lambda_{max}^{CH_3OH}$ $m\mu$ (ε): 259 (9,700) and 343 (17,800); NMR (DMSO-d_6) δ: 4.13 (2H, d, J=5 Hz, CH_2), 6.95—8.84 (3H, ABX type pattern, J_{ortho}=9.5 Hz, J_{meta}=3 Hz, aromatic), 7.38 and 7.66 (2H, D_2O exchangeable, $CONH_2$) and 9.06 (1H, t, J=5 Hz, D_2O exchangeable, NH). *Anal.* Calcd. for $C_8H_8O_5N_4$: C, 40.00; H, 3.36; N, 23.33. Found: C, 39.83; H, 3.31; N, 23.05.

B. From N-(2,4-Dinitrophenyl)glycine (16)[9]——A solution of 1.3 g of ethyl chloroformate in 3 ml of tetrahydrofuran was added with stirring to an ice-cooled mixture of 2.41 g of **16** and 1.21 g of triethylamine in 20 ml of tetrahydrofuran. After 5 min, the resulting mixture was added with vigorous stirring to 20 ml of ice-cooled 28% aq. ammonia and to this was added 500 ml of water. The precipitate obtained by filtration was subjected to chromatography on 50 g of silica gel eluting with chloroform and then with ethyl acetate. The first fraction gave 0.51 g of ethyl N-(2,4-dinitrophenyl)glycinate, mp 140—141°. An analytical sample was recrystallized from ethanol, mp 143—144° (lit.[13] mp 142—144°). IR ν_{max}^{Nujol} cm^{-1}: 3328, 3095, 1743, and 1622. NMR (acetone-d_6): 1.29 (3H, t, J=7 Hz, CH_2CH_3), 4.24 (2H, q, J=7 Hz, CH_2Me), 4.43 (2H, d, J=5 Hz, CH_2CO), 7.08—8.93 (3H, ABX type pattern, J_{ortho}=9.5 Hz, J_{meta}=2.7 Hz, aromatic) and 8.9 (1H, D_2O exchangeable, NH). *Anal.* Calcd. for $C_{10}H_{11}O_6N_5$: C, 44.61; H, 4.12; N, 15.61. Found: C, 44.41; H, 4.19; N, 15.37.

The second fraction gave 0.10 g of 2,4-dinitroaniline and the third, 0.25 g of **15**, mp 223—224°, identical with the sample obtained in A by mixture melting point, IR and TLC.

13) S. Passeron and G.A. Brieux, *Bull. Soc. Chim. France*, **1963** 35.

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Studies on Chromophore Groups of Streptothricin Group Antibiotics by Optical Rotatory Dispersion and Circular Dichroism

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In the course of searching for antibiotics, streptothricin group antibiotics such as racemomycins,[2] yazumycins,[3] containing β-lysine in their molecule, and new-type antibiotics such

1) Location: *1-14 Bunkyo-machi, Nagasaki, 852, Japan.*
2) H. Taniyama, Y. Sawada, and T. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **19**, 1627 (1971).
3) H. Taniyama, Y. Sawada, and T. Kitagawa, *J. Antibiotics*, **24**, 390 (1971)