Synthesis of Cyclopenin and Glycosminine from Phenylpyruvic Acid

Richard P. Rhee and James D. White*

Department of Chemistry, Oregon State University, Corvallis, Oregon 97331

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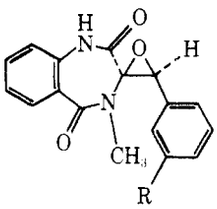
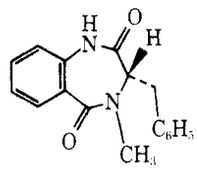
Condensation of ethyl phenylalaninate (**6**) with anthranilic acid gave **7**, which underwent cyclization to **8**. Condensation of anthranilamide (**11**) with phenylpyruvic acid, on the other hand, afforded quinazolinone **14**. The latter was converted to its methyl ester **15**, which gave **16** upon heating. Treatment of **15** with piperidine furnished **17** which, after heating in the presence of acetic acid, afforded glycosminine (**18**). Condensation of *o*-nitrobenzamide with phenylpyruvic acid yielded **20**, together with **21**. Ester **23** was converted to its *N*-methyl derivative **25**, bringing this pathway into convergence with a previous route to cyclopeptine (**4**) and cyclopenin (**1**).

Biosynthetic studies by Luckner have established that cyclopenin (**1**) and cyclopenol (**2**), metabolites of *Penicillium cyclopium* Westling, incorporate anthranilic acid and phenylalanine efficiently.[1] Supporting evidence[2] for a pathway which proceeds via the cyclic dipeptide **3** (cyclopeptine) and/or its 3,10-dehydro derivative **4** comes from (1) isolation of **3** from cultures of *P. cyclopium*,[3] and (2) the purification of a dehydrogenase which converts **3** reversibly to **4**.[4] Subsequent biological oxidation of **4** leads to **1**, which has been shown[5] to undergo an enzymatic meta hydroxylation to **2**.



1, R = H
2, R = OH



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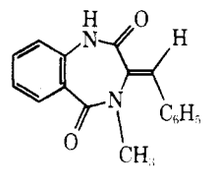

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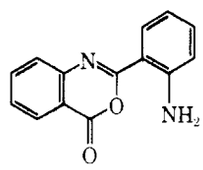
In previous syntheses of **1** and **2**,[6] **4** and its *m*-hydroxyphenyl analogue were prepared along nonbiogenetic lines and, upon epoxidation, afforded cyclopenin and cyclopenol, respectively. Intrigued by the possibility of simulating the biogenesis of **1** and related metabolites based on dehydrophenylalanine,[7] we have studied the condensation of anthranilic acid derivatives with phenylalanine and phenylpyruvic acid.

Attempts to bring about a reaction between anthranilic acid and phenylalanine in the presence of DCC in acetonitrile were unsuccessful. The sole product was the self-condensation product (**5**) of anthranilic acid.[8] However, when the ethyl ester



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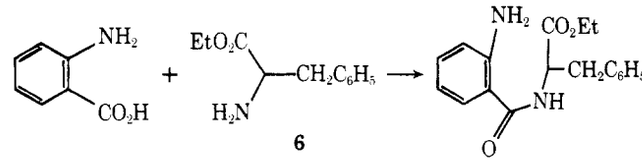
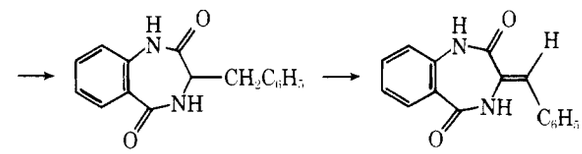
6 of phenylalanine was treated with anthranilic acid, a smooth cross-condensation led to hippuric ester **7**. Closure of **7** to the benzodiazepin **8** was effected by a mixture of piperidine and methanol at reflux. The formation of cyclic dipeptide **8** was readily apparent from the carbonyl bands in its infrared spectrum, which are typical of 1,4-benzodiazepin-2,5-diones of this type.[6,9] A further characteristic of the transformation of **7** to **8** is the upfield shift of the C-3 proton, apparently reflecting the preference of the hetero ring in **8** for a boat-like conformation (enhanced amide resonance) which places this proton on the periphery of the shielding zone of the benzo ring.

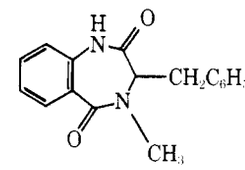
The precursor incorporation results of Luckner[2-5] imply



6 **7**



8 **10**



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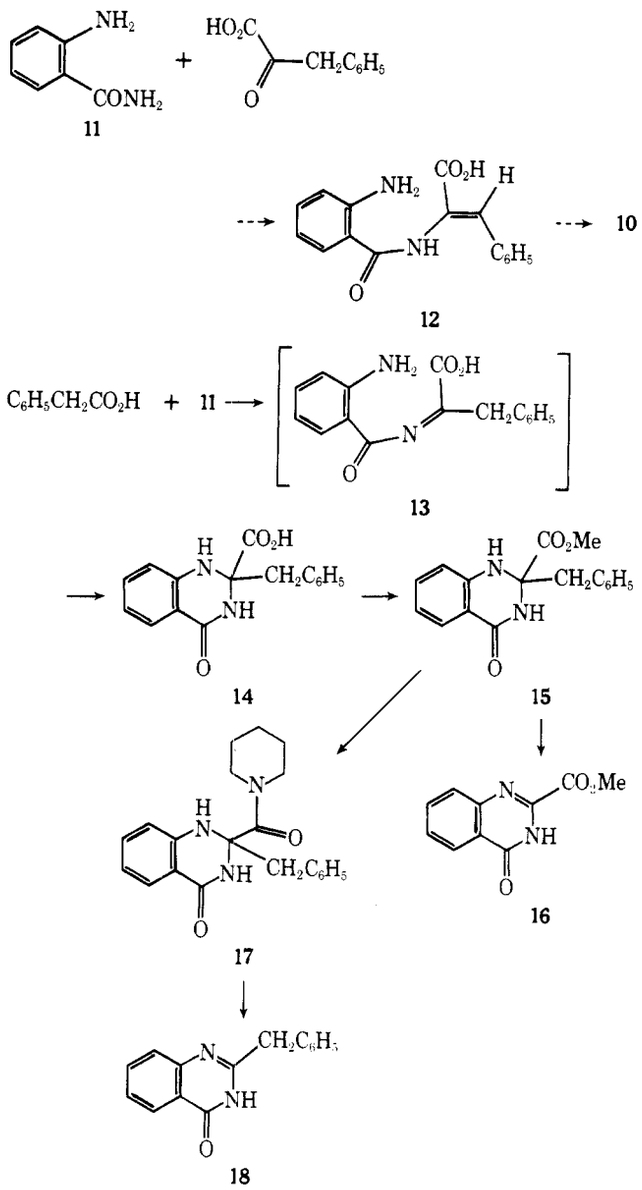
that the biosynthetic pathway to cyclopenin proceeds from **8** to the N(4)-methylated derivative (cyclopeptine) **9**. However, the 3*S* enantiomer of **8**, when treated with a purified enzyme preparation (cyclopeptine dehydrogenase) from *P. cyclopium*, was converted to nordehydrocyclopeptine (**10**) at 60% of the rate at which (3*S*)-**3** is transformed to natural cyclopeptine (**4**),[4] indicating only modest preference by the enzyme for the N-methylated substrate. Efforts directed toward N(4) methylation of **8** in the presence of base failed to yield **9** selectively, affording both O- and N-alkylated products. Furthermore, neither anthranilic acid nor its ester gave any condensation product with esters of *N*-methylphenylalanine. Significantly, it has been found that *N*-methylphenylalanine is not incorporated into cyclopenin.[2]

An alternative view of cyclopenin biosynthesis, which is not inconsistent with Luckner's results and which can be extended conceptually to other secondary metabolites derived formally from α,β-dehydroamino acids, involves transamination of an α-keto acid.[10] In the present case, phenylpyruvic acid could undergo *transamidation* with anthranilamide (**11**) to enamide **12**, which might then dehydrate to **10** (Scheme I).[11] Kirby and Narayanaswami[12] have recently shown that [3-^{3}H]phenylalanine is incorporated into cyclopenin with a loss of tritium label consistently in excess of 50%, and they suggested that conversion of phenylalanine to phenylpyruvic acid (and hence its enol) plays a significant role in the biosynthesis of **1**.

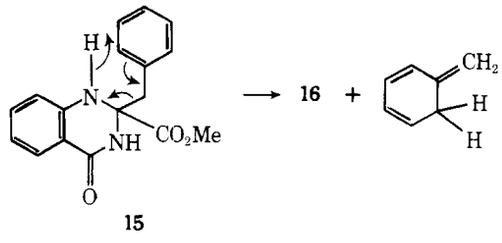
When an equimolar mixture of phenylpyruvic acid and anthranilamide (**11**) was heated in benzene, a crystalline condensation product was formed in 93% yield. Spectral data ruled out the expected structure **12** and, with the appearance of an infrared band corresponding to a *saturated* carboxylic acid, suggested the quinazolinone **14**. The latter can be readily

Scheme I



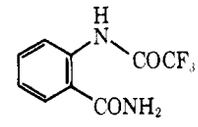
explained, either by assuming initial condensation to give acylimine **13**, which undergoes an internal, conjugate addition by the aromatic amino group, or by the alternate pathway involving a reaction between phenylpyruvic acid and the aromatic amino function of **11**, followed by addition of the amide nitrogen to the resulting imine. Esterification of **14** with diazomethane or with methanol containing sulfuric acid furnished **15**. This ester, upon exposure to refluxing xylene for 48 h, underwent a remarkably facile elimination of toluene to furnish the aromatic quinazolinone derivative **16** in good yield. A rationale for this elimination process is the retro-ene pathway depicted below.[13]

In contrast, the reaction between ester **15** and piperidine in methanol afforded the *N*-acyl derivative **17** which, upon treatment with refluxing acetic acid, yielded crystalline glycosminine (**18**). The latter is a minor alkaloid of the Indian



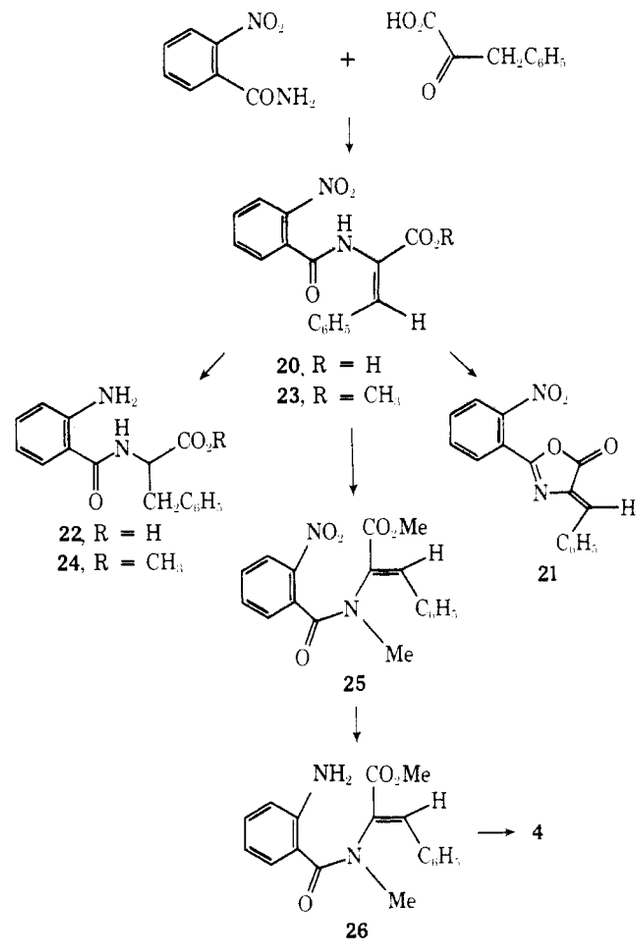
medicinal plant *Glycosmis arborea* (Roxb.)[14] and, although its biogenesis has not been investigated, a plausible origin is the condensation of anthranilic acid or a derivative with phenylalanine (or phenylpyruvic acid), followed by oxidative decarboxylation.

Since the reaction of **11** with phenylpyruvic acid led to a quinazolinone rather than the anticipated benzodiazepine **10**, it was reasoned that modification of the aromatic amino group of **11** in a way that would suppress cyclization of **13** to **14** might permit a subsequent dehydration to the cyclic dipeptide **10**. *N*-Trifluoroacetylanthranilamide (**19**) was prepared,[15] but



underwent intramolecular condensation to 2-trifluoromethyl-3,4-dihydro-4-quinazolinone in preference to reaction with phenylpyruvic acid. On the other hand, when a mixture of *o*-nitrobenzamide and phenylpyruvic acid in toluene containing *p*-toluenesulfonic acid was heated, a crystalline solid was precipitated which was readily identified as the desired benzylidenehippuric acid **20** by comparison with material prepared by an alternate route[6a] (Scheme II). Prolonged ex-

Scheme II



posure of these reactants to heat in the presence of acid led to increasing amounts of the oxazolone **21**, derived from **20** by dehydration.

Attempts to selectively reduce the nitro group of **20** by catalytic hydrogenation were unsuccessful, and led uniformly to the saturated derivative **22**. A similar outcome prevailed in the catalytic hydrogenation of ester **23**, prepared from

20 and diazomethane, which gave **24.** Since it was known from earlier studies[6a] that the *N*-methyl derivative (**25**) of **23** underwent selective hydrogenation of the nitro function, the amide **23** was converted to its salt with sodium hydride in DMF and then treated with methyl iodide. The resulting nitro amide **25** was hydrogenated to the amine **26,** which underwent condensation in methanol–piperidine at reflux to dehydrocyclopeptine (**4**). The latter has been previously converted to cyclopenin (**1**) by epoxidation.[6] The diminished reactivity of the double bond in **25** toward hydrogen, as compared with **23,** is attributed to increased steric bulk in the *N*-methyl compound, which forces the cisoid benzamide and phenyl substitutents to rotate out of coplanarity with resulting obstruction of the π system.

In conclusion, synthesis of the benzo-1,4-diazepin-2,5-dione nucleus characteristic of cyclopenin (**1**) and related metabolites via transamidation of phenylpyruvic acid with a benzamide derivative is possible when an ortho amino group in the amide is masked. When this group is free, as in anthranilamide, condensation to give the 2-benzyl-3,4-dihydro-4-quinazolinone system, common to the alkaloids arborine[16] and glycosminine (**18**), takes place.

Experimental Section

Melting points were determined on a Buchi melting-point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer Model 727B spectrophotometer. Nuclear magnetic resonance (NMR) spectra were obtained on Varian Associates EM-360 and HA-100 spectrometers. Peak positions are given in parts per million (δ) downfield from the internal standard Me_4Si. The abbreviations s, d, t, q, and m refer to singlet, doublet, triplet, quartet, and multiplet, respectively. The coupling constant (J) is given in hertz. Mass spectra were determined by Dr. Susan Rottschaefer, Department of Chemistry, University of Oregon, using a CEC-103B spectrometer. The abbreviation M^+ refers to the molecular ion.

Ethyl 2-Benzyl-*o*-aminohippurate (7). A mixture of 1.00 g (6.06 mmol) of ethyl phenylalaninate, 0.83 g (6.06 mmol) of anthranilic acid, and 1.44 g (7.0 mmol) of *N,N'*-dicyclohexylcarbodiimide in 15 mL of acetonitrile was stirred for 4 h. A few drops of acetic acid was added and the precipitated dicyclohexylurea was filtered off. The filtrate was taken up in ethyl acetate and washed once with 1 N hydrochloric acid, twice with saturated potassium bicarbonate, and once with saturated brine. The organic phase was dried (Na_2SO_4) and the solvent was removed in vacuo. The residue was chromatographed on 30 g of Merck silica gel, eluting with benzene, to give 1.52 g (80%) of **7** as an oil: IR (film) 3550, 3400, 1745, 1650, 1620, 1590, 1510 cm^{-1}; NMR ($CDCl_3$) δ 1.21 (3 H, t, J = 7), 3.20 (2 H, d, J = 6), 4.19 (2 H, q, J = 7), 4.99 (1 H, q, J = 6), 5.43 (3 H, br, disappears on addition of D_2O), 6.60 (3 H, m), 7.02 (1 H, m), 7.36 (5 H, br s).

3-Benzyl-3,4-dihydro-1*H*-1,4-benzodiazepine-2,5-dione (8). A mixture of 1.50 g (5.0 mmol) of **7** in 10 mL of methanol and 10 mL of piperidine was heated under reflux for 24 h. The solvent was evaporated in vacuo and the residue was crystallized from acetone to give 0.51 g (38.5%) of **8:** mp 270–272 °C; IR (Nujol) 3200 (br), 1675, 1655 cm^{-1}; NMR (CF_3CO_2D) δ 2.93 (2 H, d, J = 6), 4.00 (1 H, t, J = 6), 6.9–7.7 (11 H, br); m/e 266 (m^+). An analytical sample was prepared by sublimation at 200 °C (0.01 mm).

Anal. Calcd for $C_{16}H_{14}N_2O_2$: C, 72.17; H, 5.30; N, 10.52. Found: C, 72.18; H, 5.42; N, 10.52.

2-Benzyl-1,2,3,4-tetrahydro-4-oxoquinazoline-2-carboxylic Acid (14). A mixture of 2.00 g (12.2 mmol) of phenylpyruvic acid and 1.80 g (13.2 mmol) of anthranilamide in 100 mL of benzene was heated under reflux for 3 h. Water was collected from the reaction mixture in a Dean–Stark trap. During the reaction a colorless, crystalline deposit formed. After cooling the mixture, the crystalline mass was filtered to give 3.20 g (93%) of virtually pure **14:** mp 182–189 °C; IR (Nujol) 3340, 3295, 1720, 1637, 1612 cm^{-1}; NMR (CD_3SOCD_3) δ 3.2 (2 H, s), 6.4–7.7 (10 H, m), 8.25 (1 H, br s); m/e 282 (M^+).

Methyl 2-Benzyl-1,2,3,4-tetrahydro-4-oxoquinazoline-2-carboxylate (15). A solution of 0.19 g (0.68 mmol) of **14** in 25 mL of methanol containing 2 drops of concentrated sulfuric acid was heated under reflux for 4 h. The mixture was concentrated to a small volume in vacuo and was taken up into ethyl acetate. This solution was washed with saturated sodium bicarbonate and water, and dried ($MgSO_4$). Removal of the solvent in vacuo left a solid residue which was crystallized from acetone–petroleum ether to give 0.145 g (72%) of **15:** mp 182–183 °C; IR (Nujol) 3350, 3175, 1720, 1675, 1620 cm^{-1}; NMR (CD_3COCD_3) δ 3.28 (2 H, s), 3.64 (3 H, s), 6.60–7.78 (11 H, m); m/e 296 (M^+).

Anal. Calcd for $C_{17}H_{16}N_2O_3$: C, 68.91; H, 5.44; N, 9.45. Found: C, 68.78; H, 5.47; N, 9.39.

Methyl Quinazolin-4(3*H*)-one-2-carboxylate (16). A solution of 0.068 g (0.23 mmol) of **15** in 5 mL of xylene was heated under reflux for 48 h. After cooling, the mixture was diluted with ether and the crystalline product was collected by filtration. Recrystallization from acetone–petroleum ether yielded 0.025 g (54%) of **16:** mp 209–210 °C (lit.[17] 203–204 °C); IR (Nujol) 3135–3125, 1730, 1655, 1605 cm^{-1}; NMR ($CDCl_3$) δ 4.12 (3 H, s), 7.56–8.46 (5 H, m); m/e 204 (M^+).

2-Benzyl-2-piperidinoxo-1,2,3,4-tetrahydroquinazolin-4-one (17). A solution of 0.40 g (1.35 mmol) of **15** and 5.16 g (60 mmol) of piperidine in 12 mL of dry methanol was heated under reflux for 24 h. The solvent was removed in vacuo and the residue was crystallized from acetone to give 0.39 g (83%) of **17:** mp 186–189 °C; IR (Nujol) 3225, 3160, 1650, 1615 cm^{-1}; NMR (CD_3SOCD_3) δ 1.58 (6 H, br s), 2.90 (2 H, s), 2.97 (4 H, br s), 6.4–7.5 (10 H).

Anal. Calcd for $C_{21}H_{23}N_3O_2$: C, 72.18; H, 6.63; N, 12.03. Found: C, 72.04; H, 6.77; N, 11.81.

2-Benzylquinazolin-4(3*H*)-one (Glycosminine, 18). A solution of 1.00 g (2.97 mmol) of **17** in 13 mL of glacial acetic acid was heated under reflux for 4 h. After the mixture had cooled, the crystalline precipitate was filtered and washed with water. Recrystallization from ethanol afforded 0.30 g (43%) of **18:** mp 250–253 °C (lit.[14] 249 °C); IR (Nujol) 1680, 1625 cm^{-1}; NMR (CD_3SOCD_3) δ 3.94 (2 H, s), 7.3–8.2 (10 H); m/e 236 (M^+).

***trans*-2-Benzylidene-*o*-nitrohippuric Acid (20).** A mixture of 6.43 g (39 mmol) of *o*-nitrobenzamide, 9.50 g (58 mmol) of phenylpyruvic acid, and 15.0 g (77 mmol) of *p*-toluenesulfonic acid in 300 mL of toluene was heated under reflux for 4 h. The solvent was concentrated to a small volume in vacuo, and the solid residue was triturated with ether, filtered, and washed with chloroform until colorless. Recrystallization of the collected solid from acetone–petroleum ether gave 5.59 g (47%) of **20:** mp 235–237 °C (lit.[6a] 235–237 °C). This material was identical with **20** prepared by a previously described method.[6a]

The filtrate, after removal of solvent in vacuo, deposited an orange-colored solid which was taken up into hot ethanol. Crystallization at 5 °C overnight gave 2.63 g (23%) of *trans*-2-(*o*-nitrophenyl)-4-benzylidene-3-oxazol-5-one (**21**): mp 147–148 °C (lit.[6a] 151–152 °C). This material possessed chromatographic and spectral (IR, NMR) properties identical with those of authentic material.[6a]

Methyl *trans*-2-Benzylidene-*o*-nitrohippurate (23). To a solution of 0.825 g (2.65 mmol) of **20** in 10 mL of anhydrous methanol was added an excess of diazomethane in ether. After 1 h diazomethane and the solvent were removed by a water aspirator to leave a yellow, crystalline residue. This was taken up into a small volume of acetone, decolorized with charcoal, and recrystallized by addition of petroleum ether to give 0.75 g (87%) of **23:** mp 135–137 °C (lit.[6a] 141.5–143 °C).

Methyl 2-Benzyl-*o*-aminohippurate (24). A mixture of 0.20 g (0.62 mmol) of **23** and 45 mg of 10% palladium on charcoal in 10 mL of ethyl acetate was hydrogenated at atmospheric pressure. The catalyst was removed by filtration and the filtrate was concentrated to a colorless oil. Thin-layer chromatography showed this to be pure **24:** yield 0.18 g (100%); IR (film) 3450–3345, 1730, 1640 cm^{-1}; NMR ($CDCl_3$) δ 3.23 (2 H, d, J = 6), 3.78 (3 H, s), 5.03 (1 H, q, J = 6); m/e 298 (M^+).

An analogous procedure was followed for hydrogenation of **20** and afforded **22** (100%) as a colorless oil: IR (Nujol) 3500 (br), 1715, 1670, 1630 cm^{-1}; NMR (CD_3SOCD_3) δ 3.10 (2 H, d, J = 6), 4.55 (1 H, q, J = 6).

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Registry No.—**1,** 20007-87-8; **6,** 3081-24-1; **7,** 32771-73-6; **8,** 24919-39-9; **14,** 63569-80-2; **15,** 63569-81-3; **16,** 63569-82-4; **17,** 63569-83-5; **18,** 4765-56-4; **20,** 25673-46-5; **22,** 63569-84-6; **23,** 25673-45-4; **24,** 63569-85-7; phenylpyruvic acid, 156-06-9; anthranilic acid, 118-92-3; anthranilamide, 88-68-6; piperidine, 110-89-4; *o*-nitrobenzamide, 610-15-1.

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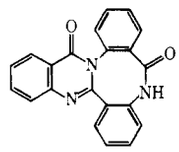

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Synthetic Approaches to Adriamycin. 2. Degradation of Daunorubicin to a Nonasymmetric Tetracyclic Ketone and Refunctionalization of the A Ring to Adriamycin

Thomas H. Smith,* Allan N. Fujiwara, William W. Lee, Helen Y. Wu, and David W. Henry

Bio-Organic Chemistry Department, Stanford Research Institute, Menlo Park, California 94025

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A synthesis of adriamycin (**2**) via elaboration of the functionalities at the 7 and 9 positions of the nonsymmetric tetracyclic ketone **5** is described. Daunorubicin (**1**) was degraded in high yield to **5** by a three-step procedure. Addition of HCN to **5** afforded the cyanohydrin **18**. The 9-OH was protected by conversion to the THP ether **19**, which afforded (±)-7-deoxydaunomycinone (**20**) upon reaction with excess MeMgI followed by acid workup. Model studies employing β-tetralones **6a** and **6b** as substrates showed this sequence to be superior to several other potential methods of side-chain elaboration. Stepwise stereo- and regiospecific hydroxylation of the 7 and 14 positions of 7-deoxydaunomycinone (**3**) afforded adriamycinone (**29**). By a minor modification of the 7-hydroxylation procedure, 7-epidaunomycinone (**27**) is obtained as the major product. The 14-OH was protected by conversion to the *p*-anisyldiphenylmethyl ether **30**. This was condensed with the protected 1-chlorodaunosamine derivative **36** under Koenigs–Knorr conditions to afford adriamycin (**2**) after deprotection.

The anthracycline antibiotics daunorubicin (**1**)[2] and adriamycin (**2**)[3] are clinically useful antineoplastic agents, with adriamycin having an especially broad spectrum of activity. However, chemotherapy employing these drugs is hampered by a number of undesirable side effects, the most serious being dose-related cardiotoxicity.[3b,4] As part of this laboratory's ongoing efforts to prepare anthracyclines having improved therapeutic properties, the possibility of developing a practical total synthesis of **2** was investigated. We now report the results of those studies.

Due to the important biological activities of **1** and **2** considerable interest has been shown in their synthesis and several aspects have been explored.[1,5] Since practical syntheses of the daunosamine sugar moiety[5c,f] and a circuitous synthesis of the aglycone[5b] had been reported, the formal total synthesis of **1** was completed in 1974 with the report of stereospecific coupling of the aglycone and sugar moiety.[1c] In this paper, we describe the elaboration of the tetracyclic nonasymmetric ketone **5** to adriamycin (**2**). In our work, **5** was obtained by degradation of daunorubicin, but a total synthesis of **5** which was subsequently elaborated to (±)-daunomycinone was recently described by Kende et al.[5a] via a Diels–Alder sequence, an approach that has received much recent attention.[6]

Treatment of daunorubicin (**1**) with sodium dithionite (Scheme I) resulted in reductive cleavage of the glycoside bond to afford 7-deoxydaunomycinone (**3**) in quantitative yield. Reduction of the 13-carbonyl was achieved with $LiAl(t\text{-}BuO)_3H$ in THF to afford the 13-dihydro compound **4** as a diastereomeric mixture in 80% yield. Periodate cleavage of the glycol was unusually slow, requiring 2 equiv of $NaIO_4$ at 23 °C for 16 h to produce a 99% yield of **5** with 71% conversion of **4**.

Scheme I