169–171°; ir ($CHCl_3$) 1770, 1670 cm^{-1}; uv λ_{max} (EtOH) 215 mμ (ε 13,700); $[\alpha]^{20}D$ −73.8° (*c* 1.35, $CHCl_3$).

Anal. Calcd for $C_{23}H_{34}O_4$: C, 73.76; H, 9.5. Found: C, 73.85; H, 9.23.

Allowing this material to stand in moist Me_2CO caused gradual precipitation of the crystalline product **5**: mp 246–250°; $[\alpha]^{25}D$ −123.4° (*c* 0.95); ir (Nujol) 1750 cm^{-1}; uv λ_{max} (EtOH) 217 mμ (ε 13,350).

Anal. Calcd for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.57; H, 8.76.

17β-Hydroxy-3-oxo-5α-androstan-$\Delta^{2,\alpha}$-acetic Acid (7).—A solution of 5.4 g (0.02 mol) of 5α-androstanolone in 100 ml of MeOH and 100 ml of H_2O was treated with 1.6 g of NaOH followed by 7.4 g of commercial 40% glyoxylic acid solution (Eastman Kodak) in 50 ml of MeOH. The milky suspension cleared upon addition of the glyoxylic acid and gradually a gelatinous precipitate formed. The mixture was refluxed for 3 hr. The cooled solution was diluted with H_2O and extracted with Et_2O to remove unreacted starting material. The aqueous layer was acidified to pH 5 with a glacial AcOH and the residual Et_2O was removed by bubbling in a nitrogen stream. A white granular precipitate formed rapidly after most of the residual Et_2O had been removed. The precipitate (5 g) was collected and recrystallized: mp 221–223° dec (MeOH–H_2O); uv λ_{max} (EtOH) 240 (ε 10,000) and 230 mμ (shoulder). The nmr spectrum showed olefinic absorption at δ 6.30; $[\alpha]^{25}D$ 114.3° (*c* 1.05, EtOH).

Anal. Calcd for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.90; H, 8.90.

Sodium Borohydride Reduction of 7.—A solution of **7** (0.5 g) in 15 ml of absolute MeOH was cooled to 0° and treated with 15 ml of an aqueous solution of 0.3 g of $NaBH_4$. The ice bath was removed after 30 min, and stirring was continued for 30 min at room temperature. The reaction was refluxed for 30 min and then cooled. After the dropwise addition of 25 ml of 25% NaOH solution, the solvent was removed *in vacuo* and the residual solid was slurried with H_2O. Acidification of the alkaline mixture gave a white precipitate of **8** which was crystallized from MeOH (0.35 g): mp 278–282° dec; uv λ_{max} (EtOH) 220 mμ (ε 20,000); $[\alpha]^{25}D$ −3.6° (*c* 0.684, $CHCl_3$); nmr (DMSO) δ 5.88 (olefinic proton).

Anal. Calcd for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.02; H, 9.53.

This dihydroxy acid was further characterized by conversion into its diacetate by dissolving 0.3 g in 30 ml of 1:1 pyridine–Ac_2O and allowing the solution to stand overnight at room temperature. Water (20 ml) was added very carefully with cooling, and the solution was heated for 2 hr on a steam bath and added to 100 ml of ice–water. The precipitate **9** was collected and recrystallized from MeOH (0.25 g): mp 206–210°; $[\alpha]^{25}D$ −2.90° (*c* 0.831, $CHCl_3$); uv λ_{max} (EtOH) 222 mμ (ε 20,000).

Anal. Calcd for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.41; H, 8.45.

3β,17β-Dihydroxy-5α-androstan-2β-acetic Acid 3,17-Diacetate (10).—A solution of 10.0 g of **9** in 250 ml of HOAc containing 1.0 g of PtO_2 was hydrogenated in a Parr shaker until the rate of hydrogenation diminished to a low level. The product was isolated, and its uv spectrum showed 55% starting material still remaining. This material was diluted with HOAc (250 ml), fresh catalyst was added (0.6 g), and the mixture was hydrogenated at atmospheric pressure until the level of starting material was reduced to 10–15% (assayed by uv). The product was isolated as an oil, dissolved in 3:1 petroleum ether (30–60°)–Et_2O, and chilled. An amorphous solid was precipitated (2.33 g) which was recrystallized from Me_2CO–petroleum ether to give a crystalline solid **10**, mp 220–224°, which was identical with 3β,17β-dihydroxy-5α-androstan-2β-acetic acid 3,17-diacetate obtained by us in another study.[14] The mother liquors were concentrated further to give an amorphous solid, mp 103–107° (7.3 g). When a sample of the latter was hydrolyzed and lactonized (see accompanying procedure for preparation of **12**), it was judged to be a mixture of epimers at C-2. The 2β isomer was present in approximately 60–70% as indicated by the nmr signal of the C-19 methyl group; the chemical shifts of the two epimers occur at 54 and 58 cps, with the latter signal predominating slightly. Attempts at purifying this mixture further were not successful.

3β,17β-Dihydroxy-5α-androstan-2β-acetic Acid (11).—A solution of **10** (2 g, 0.46 mmol) was dissolved in 270 ml of H_2O and 30 ml of 2 *N* methanolic KOH. After standing for 4 hr at room temperature, the reaction mixture was concentrated to half volume and extracted with three 100-ml portions of Et_2O. The extract was dried over $MgSO_4$ and evaporated to give 43 mg of starting material. The basic layer was acidified to pH 2 and extracted with Et_2O. The dried extract was evaporated to give 1.51 g of **11** as a white solid which was recrystallized from Me_2CO–petroleum ether: mp 177–180° (resolidifies, melts at 202–204°).

Anal. Calcd for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78. Found: C, 72.06; H, 9.71.

Lactonization of 3β,17β-Dihydroxy-5α-androstan-2β-acetic Acid (11).—A solution of 0.68 g of **11** in 75 ml of C_6H_6 and 50 mg of *p*-toluenesulfonic acid monohydrate was refluxed for 4 hr until tlc showed no starting material remaining. The solution was cooled and more C_6H_6 was added. After washing with saturated $NaHCO_3$ solution and drying over $MgSO_4$, the solvent was evaporated to dryness and the resulting oil was crystallized from MeOH to give lactone **12** as a white solid (0.7 g): mp 199–201° (MeOH); ir ($CHCl_3$) 1750 cm^{-1}.

Anal. Calcd for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.58; H, 9.54.

Registry No.—Glyoxylic acid, 298-12-4.

Acknowledgment.—The authors are indebted to Drs. E. Farkas and R. T. Rapala for their timely advice and criticisms during this work, as well as to the members of the physicochemical and microanalytical sections of the Lilly Laboratories for technical support.

(14) M. Debono and R. M. Molloy, *J. Org. Chem.*, in press.

N-Pyruvoylanthranilic Acid. Evidence against a Cyclol Structure[1]

PAUL F. WEGFAHRT AND HENRY RAPOPORT

Department of Chemistry, University of California at Berkeley, Berkeley, California 94720

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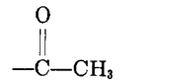
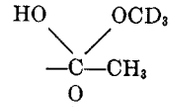
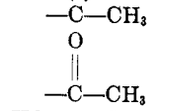
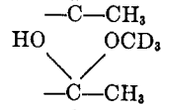
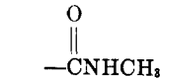
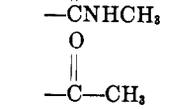
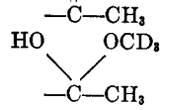
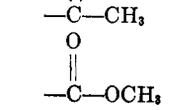
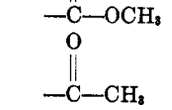
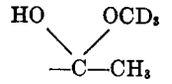
N-Pyruvoylanthranilic acid and several of its derivatives, metabolites of a number of microorganisms, were examined for the presence of a cyclol form in solution. The nmr spectra in several solvents failed to provide evidence for such a form, although a hemiketal was observed in protic solvents. Selective exchange with $H_2{}^{18}O$ at the ketone carbonyl, followed by cyclization to 1-acetyl-3-methylene-4,1-benzoxazepine-2,5-dione and subsequent ^{18}O analysis, showed complete retention of the isotope label. The previously[2] postulated cyclol intermediate, invoked to explain the nmr spectrum and formation of the benzoxazepine, is inconsistent with this evidence. An alternate mechanism for the formation of benzoxazepine is proposed.

Recent investigations of bacterial and mold metabolites have uncovered several derivatives of N-pyruvoylanthranilic acid (**1a**). In *Aerobacter aerogenes*, **1a** has been proposed as an intermediate in the biosynthesis of anthranilic acid.[3] Fermentations of *Pencillium chrysogenum* and *P. notatum* were found to produce N-pyruvoylanthranilamide (**1b**).[4] A related compound, 2-(3-hydroxy-3-phenylpyruvoylamino)-N-methylben-

(1) Supported in part by the U. S. Army Research Office, Durham, N. C.
(2) F. Lingens and B. Sprössler, *Ann.*, **702**, 169 (1967).
(3) C. Ratledge, *Nature*, **203**, 428 (1964).
(4) P. J. Suter and W. B. Turner, *J. Chem. Soc., C*, 2240 (1967).

TABLE I

METHYL SIGNALS IN THE NMR SPECTRA[a] OF N-PYRUVOYLANTHRANILIC ACID AND SOME OF ITS DERIVATIVES

Compound	Solvent: DMSO-d_6	Solvent: C_5H_5N	Solvent: CD_3OD	Assignment
N-Pyruvoylanthranilic acid (**1a**)	2.44 (3)	2.06 (3)	2.49 (2)	
			1.57 (1)	
N-Pyruvoylanthranilamide (**1b**)	2.45 (3)	2.09 (3)	2.47 (2)	
			1.55 (1)	
N-Pyruvoylanthranilic acid N-methylamide (**1c**)	2.88 (3)		2.90 (3)	
	2.43 (3)		2.45 (2)	
			1.55 (1)	
Methyl N-pyruvoylanthranilate (**1d**)	3.90 (3)	3.33 (3)	3.92 (3)	
	2.45 (3)	2.07 (3)	2.47 (2)	
			1.56 (1)	

[a] δ values, relative to internal TMS (δ 0), followed by number of protons in parenthesis.

zamide (**2**) has been proposed as an intermediate in the rearrangement of cyclopenin (**3**) to viridicatin (**4**).[5]

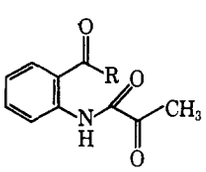
Lingens and Sprössler[2] reported the synthesis of N-pyruvoylanthranilic acid (**1a**) and concluded that in methanol solution this compound existed, in part, as the seven-membered cyclol, **5a**. They subsequently invoked this form as an intermediate to explain the facile cyclization of N-pyruvoylanthranilic acid to 1-acetyl-3-methylene-4,1-benzoxazepine-2,5-dione (**7**) in acetic anhydride–pyridine. The supporting evidence for the cyclol postulate consisted of a time-dependent shift in the ultraviolet (uv) absorption in methanol and the separation of the methyl peak in the nmr spectrum in deuteriomethanol into two peaks at δ 2.5 and 1.6 in a ratio of 2:1, assigned to the methyl ketone and cyclol forms, respectively. Several model compounds, including the dimethyl ketal of **1a**, were prepared to confirm the nmr positions. Interestingly, the cyclol methyl ether, **5b**, was not observed in the preparation of the dimethyl ketal.

Our interest in the mold metabolite cyclopenin[6] prompted us to investigate this phenomenon. If a similar cyclization of the amide analog of **1a** could be accomplished, then an alternate synthetic route and a possible biosynthetic path to cyclopenin could be envisioned. There is sufficient precedent to believe that amide nitrogen–carbonyl interaction could produce a similar cyclol.[7]

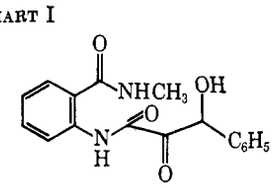
(5) Y. S. Mohammed and M. Luckner, *Tetrahedron Lett.*, 1953 (1963).
(6) H. Smith, P. Wegfahrt, and H. Rapoport, *J. Amer. Chem. Soc.*, **90**, 1668 (1968).
(7) G. I. Glover, R. B. Smith, and H. Rapoport, *ibid.*, **87**, 2003 (1965).

Nmr Studies.—The compounds **1a–1d** (Chart I) were investigated by nmr at 60 MHz in a variety of solvents, to clarify the nature of the interaction. The

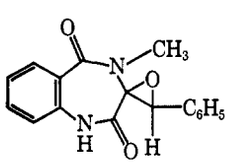
CHART I



1a, R = OH
b, R = NH_2
c, R = $NHCH_3$
d, R = OCH_3



2



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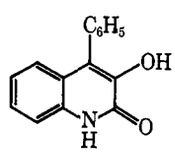
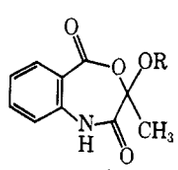
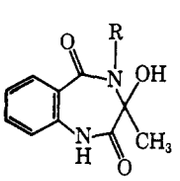


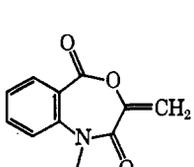
4



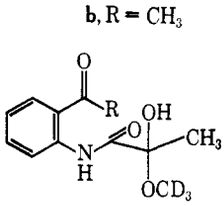
5a, R = H
b, R = CH_3



6a, R = H
b, R = CH_3

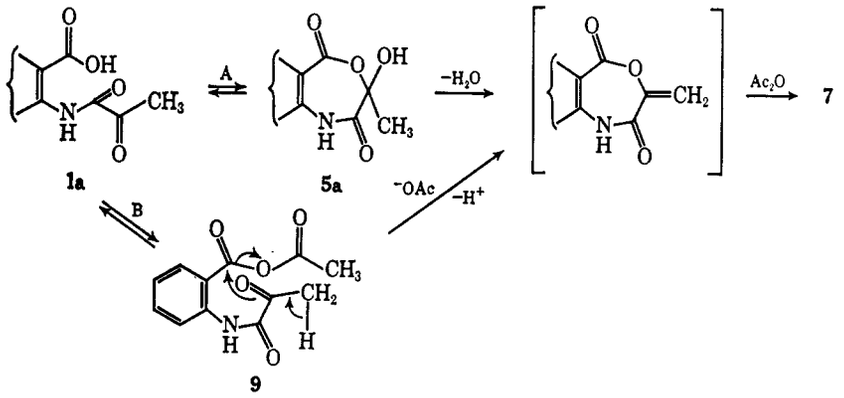


7



8a, R = OH
b, R = NH_2
c, R = $NHCH_3$
d, R = OCH_3

SCHEME I



results, shown in Table I, make it abundantly clear that what is being observed is not cyclol formation.

Splitting of the pyruvoyl methyl signal of compounds **1a–1d** was observed only in deuteriomethanol, or in other solvents only after methanol had been added. For all compounds in methanol, the positions and relative intensities of the two peaks attributable to the pyruvoyl methyl were the same. The high-field signal (δ 1.6) was previously assigned to the methyl of the hydroxylactone form **5a**,[2] *i.e.*, methyl on carbon bearing two oxygens. Such a methyl group would not be expected, on the basis of shielding effects, to have the same chemical shift as the methyl of the amide cyclols **6a** and **6b**, *i.e.*, methyl on carbon bearing oxygen and nitrogen, nor would the equilibrium amounts of amide and carboxylic acid cyclols be expected to be the same.

The strongest evidence against existence of any appreciable amount of cyclol form is found in the spectrum of the methyl ester, **1d**, a compound where cyclol formation is not possible, but where the same pattern of methyl splitting is observed. The most reasonable interpretation of the data is that in the presence of methanol the hemiketals **8a–8d** are in equilibrium with the ketone, and the position of equilibrium is independent of the carboxylate substituent.

The spectra in nonprotic solvents gave no evidence for a cyclol form in solution, although this alone is not sufficient proof that none exists. Time averaging of the two methyl signals or a very small equilibrium amount of cyclol would be undetectable by the method used. Thus the conversion of **1a** into benzoxazepine **7** still could have proceeded *via* the previously postulated cyclol **5a** followed by dehydration.

^{18}O Studies.—Inspection of the cyclol mechanism for benzoxazepine formation (path A) suggests a method for testing its validity, since the ketonic oxygen would be lost during reaction by this path. An alternate path which proceeds *via* the mixed anhydride **9** (path B) retains the ketone oxygen in the benzoxazepine ring. Selective ^{18}O exchange at the ketone carbonyl, cyclization to **7**, and ^{18}O analysis of the cyclic product would differentiate between the two paths (see Scheme I).

The apparent ease of hemiketal formation suggested that the ketone oxygen could be exchanged with $H_2{}^{18}O$ under quite mild conditions. Accordingly, **1a** was dissolved in 10:1 tetrahydrofuran–water (1.71% ^{18}O) without catalysis, and samples were withdrawn periodically over a 72-hr period. The ^{18}O content, determined by nonoxidative pyrolysis of the crystalline material and mass spectroscopy of the resulting carbon dioxide,[8] increased regularly, reached a maximum value after 24 hr, and remained constant. Since the ^{18}O content of **1a** after exchange had ceased was only 25% that of the water (Table II), only one of the four oxygens had exchanged, and that presumably was the ketonic oxygen since amides and carboxylic acids usually require more vigorous conditions for oxygen exchange.[9]

TABLE II

^{18}O ANALYSES OF N-PYRUVOYLANTHRANILIC ACIDS (**1a**) AND 2-ACETYL-3-METHYLENE-4,1-BENZOXAZEPINE-2,5-DIONE (**7**)

Compound	% ^{18}O, total[a]	Calcd % ^{18}O of ketone oxygen[b]
H_2O	1.71 ± 0.01	
1a control	0.20 ± 0.01	0.20
1a exchanged	0.57 ± 0.01	1.68 ± 0.04
7[c]	0.58 ± 0.01	1.73 ± 0.04

[a] Determined by mass spectroscopy of pyrolysate, average of two determinations. [b] Calculated assuming that ^{18}O enrichment occurred only in the ketone carbonyl oxygen; % ^{18}O (ketone) = [total ^{18}O − 0.20 (natural abundance)] × number of oxygen atoms + 0.20. [c] Prepared from **1a** − exchanged.

To establish firmly the position of the oxygen which had exchanged, N-pyruvoylanthranilic acid (**1a**) was equilibrated with 30.2% $H_2{}^{18}O$ (containing a large amount of deuterium) and the mass spectrum of this exchanged material was compared with that of the unlabeled compound (Figure 1). The molecular ion of the exchanged material had significant peaks from *m/e* 207, M^+ of the original material, to *m/e* 211 (M + 4). The M + 4 ion contains one atom of ^{18}O and two atoms of deuterium since the absence of a significant M + 5 peak implies that only one oxygen and the two hydrogens, on the amide and acid groups, are exchangeable.

The position of oxygen exchange becomes apparent on inspection of the first fragmentation peak, which occurs at *m/e* 164 (M − 43), and can be attributed to the loss of acetyl. The absence of peaks at *m/e* 167 and 168 and the intensities of the ions at *m/e* 165 and 166 show that the ^{18}O label has been lost and that only deuterium remains. Additionally, the presence of the ^{18}O in the acetyl group can be seen in the greatly enhanced ratio of *m/e* 45 to *m/e* 43 in the exchanged material over that found in the spectrum of unlabeled **1a**.

(8) C. D. Snyder and H. Rapoport, *Biochemistry*, **7**, 2318 (1968).

(9) D. Samuel in "Oxygenases," O. Hayashi, Ed., Academic Press Inc., New York, N. Y., 1962, pp 31–86.

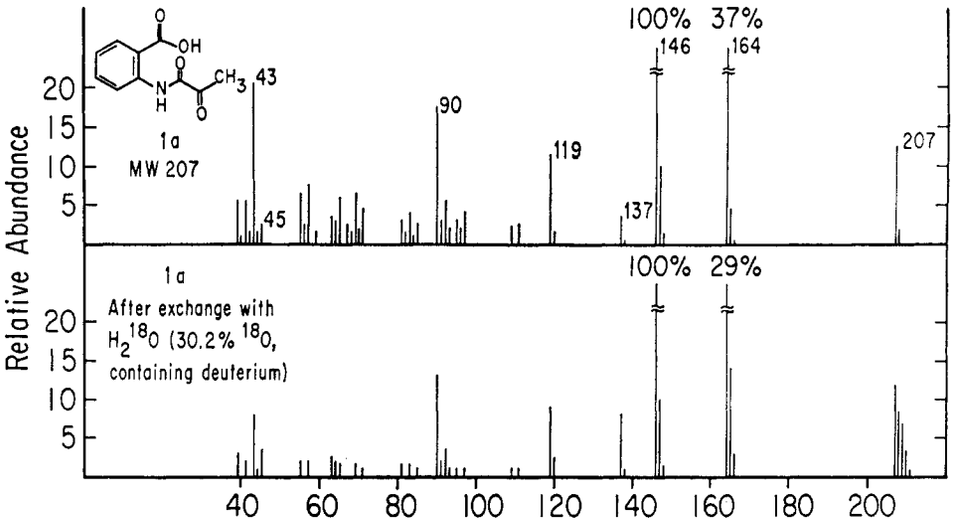
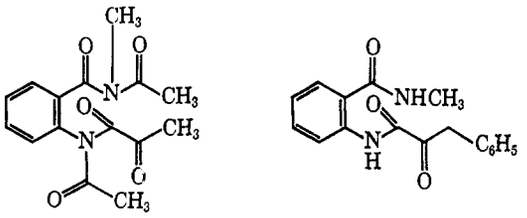


Figure 1.—Mass spectra of N-pyruvoylanthranilic acid (**1a**) before and after exchange with $H_2{}^{18}O$–$D_2{}^{18}O$.

Loss of the elements of water from the *m/e* 164 ion gives the base peak at *m/e* 146 in which the isotopic content is identical with that of the corresponding peak in the spectrum of unlabeled **1a**. This confirms that the ketone carbonyl was the sole position of ^{18}O exchange.

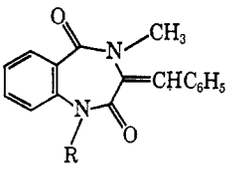
The isotopically labeled material was then converted into the benzoxazepine **7** as described.[2] Analysis of ^{18}O in the product showed complete retention of the isotopic label (Table II). Thus the cyclol intermediate (path A) cannot be contributing to benzoxazepine formation. Path B, which involves displacement of the mixed anhydride, is supported by the evidence.

Although the cyclization *via* the mixed anhydride is not applicable to the amide analogs, the possibility of cyclolization to form a benzodiazepine (**6**) is still possible. In fact, it could be argued that, with the acid **1a**, cyclization *via* path A was merely suppressed by the existence of a more favorable route. Such a route does not exist for the amide; consequently the amide **1c** was subjected to the same conditions that gave cyclization of the acid. Two compounds were isolated from the reaction mixture, which on characterization by mass and nmr spectroscopy appeared to be isomeric diacetyl derivatives of **1c**. Attempts to interconvert thermally the two materials by heating at temperatures up to 150° led only to decomposition. No attempt was made to define rigorously the structure of those materials, but they are believed to be isomers of **10**.



10

11



12a, R = H
b, R = $COCH_3$

As a final attempt to detect benzodiazepine formation *via* cyclolization of ring-open compounds, the phenylpyruvoyl analog **11** was prepared and subjected to the same cyclization conditions. Comparisons by thin layer chromatography (tlc) of the products of the reaction with authentic samples of the anticipated products **12a** and **12b**, prepared by a different route,[6] failed to give any evidence of benzodiazepine formation.

It must be concluded that there is no evidence to support the existence of a cyclol form in pyruvoylanthranilates. Molecular models indicate that the desired ring system is significantly strained, and, although the ring has been formed by closure of the 3–4 bond,[6,10] this has occurred only under irreversible conditions.

Experimental Section

Pyruvoyl Chloride.—Reagent grade thionyl chloride (12 g, 0.10 mol), 95% pyruvic acid (5.0 g, 0.05 mol), and 200 ml of anhydrous ether were boiled in a nitrogen atmosphere for 4 hr. The solvent and excess thionyl chloride were removed *in vacuo* to give 4.2 g (80%) of crude pyruvoyl chloride as a yellow oil, which was used without further purification.

N-Pyruvoylanthranilic acid (1a) was prepared as described[2] in 30% yield after seven recrystallizations: mp 194–195° (lit.[2] mp 194–195°).

N-Pyruvoylanthranilamide (1b).—The crude pyruvoyl chloride (4.2 g, 0.04 mol) in 50 ml of methylene chloride was added over 1 hr to a stirred solution of anthranilamide (5.5 g, 0.04 mol) and pyridine (3.2 g, 0.04 mol) in 200 ml of methylene chloride at 0° and stirred at room temperature for 6 hr. The resulting solution was filtered and washed three times with 1 *N* hydrochloric acid, then with 5% aqueous sodium bicarbonate and water. The solvent was evaporated and the residue was crystallized from methylene chloride–pentane: yield 3.7 g (45%); mp 180–182° (lit.[4] mp 181–184°).

N-Pyruvoylanthranilic acid N-methylamide (1c) was prepared from anthranilic acid N-methylamide and pyruvoyl chloride following the procedure used for preparing anthranilamide. Silica gel chromatography of the crude product gave 2.7 g (31% yield) after crystallization from methylene chloride–pentane: mp 142–144°; $\lambda_{max}^{CH_3OH}$ 302 nm (ϵ 5900), 241 (9600).

Anal. Calcd for $C_{11}H_{12}N_2O_3$: C, 60.0; H, 5.5; N, 12.7. Found: C, 60.2; H, 5.5; N, 12.6.

Methyl N-pyruvoylanthranilate (1d) was prepared in 64% yield from methyl anthranilate and pyruvoyl chloride: mp 111–112° (lit.[2,4] mp 111–112°).

N-Phenylpyruvoylanthranilic Acid N-Methylamide (11).—Phenylpyruvic acid (1.63 g, 0.01 mol) was dissolved in 20 ml of reagent grade thionyl chloride and warmed under nitrogen at

(10) C. Lee, *J. Heterocycl. Chem.*, **1**, 235 (1964).

50° for 3 hr. The excess reagent was removed *in vacuo* to give 1.8 g of a yellow oil. This material was dissolved in 50 ml of chloroform and added over 1 hr to a stirred solution of anthranilic acid N-methylamide (1.5 g, 0.01 mol) and pyridine (0.9 g, 0.011 mol) in 150 ml of chloroform at 0°. The solution was stirred overnight at room temperature, refluxed for 1 hr, washed with 1 *N* hydrochloric acid, 5% aqueous sodium bicarbonate, and water, and dried over sodium sulfate, and the solvent was removed *in vacuo*. Chromatography on silica gel and crystallization from ethyl acetate gave 1.2 g (41%) of **11** as fine needles: mp 182–185° dec; $\lambda_{max}^{C_2H_5OH}$ 302 nm (ε 7900), 249 (12,800); nmr (DMSO-d_6) δ 2.82 (3, d), 4.15 (2, s), 6.9–7.9 (9, m), 8.9–9.1 (2, br).

Anal. Calcd for $C_{17}H_{16}N_2O_3$: C, 68.9; H, 5.4; N, 9.5. Found: C, 69.0; H, 5.2; N, 9.4.

1-Acetyl-3-methylene-4,1-benzoxazepine-2,5-dione (7) was prepared from **1a** in acetic anhydride, by the method described,[2] in 70% yield: mp 95° (lit.[2] mp 95–96°).

Treatment of N-Pyruvoylanthranilic Acid N-Methylamide (1c) with Acetic Anhydride–Pyridine.—A solution of 0.5 g of **1c** in 5 ml of 1:1 acetic anhydride–pyridine was allowed to stand for 2 days at room temperature and then heated on a steam bath for 6 hr. The solution was diluted with ether, washed with 1 *N* hydrochloric acid, 5% aqueous sodium bicarbonate, and water, and dried over sodium sulfate. Chromatography on silica gel gave two major products: A [150 mg; mp 134–135°; M+ 304; $\lambda_{max}^{CH_3OH}$ 294, 240, 221 nm; nmr ($CDCl_3$) δ 1.59 (3, s), 1.62 (3, s), 2.10 (3, s), 2.98 (3, s), 7.0–8.0 (4, m)] and B [65 mg; mp 157–159°; M+ 304; $\lambda_{max}^{CH_3OH}$ 291, 240, 218 nm; nmr ($CDCl_3$) δ 1.65 (3, s), 1.78 (3, s), 2.00 (3, s), 3.00 (3, s), 7.1–8.2 (4, m)].

^{18}O Exchange of Ia.—Analytically pure N-pyruvoylanthranilic acid (**1a**, 200 mg) was dissolved in 10 ml of dry tetrahydrofuran in a capped serum vial, 1 ml of $H_2{}^{18}O$ (1.71% ^{18}O) was added, and the solution was allowed to stand at room temperature for 72 hr. Aliquots (5 mg), taken periodically to measure the extent of exchange, were added to pyrolysis tubes and the solvent was removed immediately under a nitrogen stream. Succeeding exchanges were run for 24 hr only. Rigorously dried solvents and glassware were used through all ^{18}O experiments. The samples (5 mg) of the material to be analyzed were dried thoroughly under high vacuum and then pyrolyzed at 500° for 4 hr as previously described.[7] The pyrolysis tube was cooled in a methanol–Dry Ice bath and the resulting gases were examined on a mass spectrometer. Since the yield of carbon monoxide was insufficient for the purposes of analysis, the carbon dioxide peak was used. Background hydrocarbons were minimal and the analysis was straightforward. The ^{18}O content of the water was determined by equilibration of a sample with CO_2 in a pyrolysis tube at 500° followed by mass spectroscopy of the gas. The isotope content of the CO_2 was calculated from formula 1

$$^{18}O\ (\%) = \frac{[46]/([44] + [45]) \times 0.96}{2 + \{[46]/([44] + [45])\}} \times 100 \qquad (1)$$

where [44], [45], and [46] are the relative intensities of the *m/e* 44, 45, and 46 peaks.

Registry No.—**1a**, 14469-11-5; **1b**, 18326-62-0; **1c**, 20452-61-3; **1d**, 13748-93-1; **11**, 20453-01-4.

The Synthesis of 1-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-18-(2,6,6-trimethyl-2-cyclohexen-1-ylidene)-3,7,12,16-tetramethyl-2,4,6,8,10,12,14,16,18-octadecanonaene and Its Rearrangement to *trans*-β-Carotene

J. D. SURMATIS, J. GIBAS, AND R. THOMMEN

Technical Development Department, Hoffmann-La Roche, Inc., Nutley, New Jersey

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A novel synthesis of 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-18-(2,6,6-trimethyl-2-cyclohexen-1-ylidene)-3,7,12,16-tetramethyl-2,4,6,8,10,12,14,16,18-octadecanonaene (**6**) and its rearrangement to *trans*-β-carotene are reported. A new type of carotenoid with a cross-conjugated keto function (**4**) was used as an intermediate for the synthesis of **6**.

The first synthesis of 1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-18-(2,6,6-trimethyl-2-cyclohexen-1-ylidene)-3,7,12,16-tetramethyl-2,4,6,8,10,12,14,16,18-octadecanonaene (**6**) was reported in 1956.[1] In spite of its structural similarity to β-carotene, very little is known about this carotenoid; it has not been found in nature nor reported to play a role in the biosynthesis of naturally occurring carotenoids. The ease with which it was transformed into β-carotene may explain its absence in natural products. By using a readily available intermediate (**1**) of an industrial vitamin A synthesis,[2,3] **6** was prepared in high yield and rearranged to *trans*-β-carotene.

A search of the literature resulted in one reference[4] describing a carotenoid with a cross-conjugated keto function. Its structure had not been fully established yet, as the work was hampered by a lack of material and published data on this type of configuration. This was an "open-ring" carotenoid belonging to a class represented by lycopene.

The C-20 diol (**1**) was oxidized with manganese dioxide[5] in methylene chloride to afford a cross-conjugated keto aldehyde, **2** (65%), as yellow crystals, mp 74°. The condensation of **2** with retinylphosphonium sulfate (**3**) resulted in a new type of keto carotenoid, **4** (60%), mp 156°. Compound **4** crystallized as dark violet hexagonal prisms from benzene–methanol and as red rhombic crystals from heptane. The color of a solution of **4** in benzene or heptane was similar to that of *trans*-β-carotene of equal concentration. Compound **4** was approximately twice as soluble in heptane as *trans*-β-carotene.

The nuclear magnetic resonance (nmr) spectrum was compatible with the structure assigned to **4** (Figure 1).

The ultraviolet spectrum of **4** showed a weaker absorption than that of *trans*-β-carotene, and the curve exhibited only one maximum (Figure 2).

The retinyltriphenylphosphonium sulfate (**3**) was obtained as a yellow crystalline monohydrate on react-

(1) O. Isler, M. Montavon, R. Ruegg, and P. Zeller, *Helv. Chim. Acta*, **39**, 454 (1956).

(2) O. Isler, A. Ronco, W. Guex, N. C. Hindley, W. Huber, K. Dialer, and M. Koffler, *ibid.*, **32**, 489 (1949).

(3) J. D. Surmatis, U. S. Patent 2,610,208 (1952).

(4) S. L. Jensen and K. Schmidt, *Arch. Mikrobiol.*, **46**, 138 (1963).

(5) Available from General Metallics Oxides Corp., Jersey City, N. J. (Manganese hydrate no. 37).