[Chem. Pharm. Bull. 16(11)2269—2273(1968)]

UDC 615.033/.034 : 547.551.42

Drug Absorption, Metabolism, and Excretion. III.[1] Synthesis of the Metabolites of Bucetin (β-Hydroxybutyro-*p*-phenetidide)

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(Received December 26, 1967)

In the previous paper,[1] the metabolism of bucetin (β-hydroxybutyro-*p*-phenetidide, Ia)[3] was investigated and it became necessary to prepare acetoacetyl-*p*-phenetidide (IIa), *p*-hydroxyacetoacetanilide (IIb), and N-(β-hydroxybutyro)-*p*-aminophenol (Ib) for the purpose of identifying the metabolites.

In the present paper, the synthesis of these compounds are described with the related facts observed during the course of the preparation.

Syntheses of Acetoacetanilides (II)

For the preparation of II, there have been reported two methods; condensation of acetoacetates with aromatic amines, and addition of diketene to aromatic amines.

The reaction of ethyl acetoacetate and aromatic amines has been studied extensively,[4] and it has been shown that the reaction at high temperature affords II, whereas β-anilinocrotonates (IV) at room temperature.[4c,d] At first, authors attempted to prepare IIa and IIb by this condensation method under heating, the former being already obtained by the similar way,[4c,d] but in either case, II was yielded accompanied with a considerable amount of respective IV and starting amines. Further, the trial to convert IV to II by heating IV in the manner described by Hauser, *et al.*[4d] gave no satisfactory results. On the contrary, it was confirmed that the condensation at low temperature produced IV without contamination of II.

The other method of II synthesis, *i.e.* the reaction of diketene with aromatic amines has been known for long.[5,6] IIa[5c] and IIb[5d] were prepared already in this way, though the detail of the latter including the melting point was not available. Authors performed the reaction in benzene for IIa preparation and in the mixed solvent of methanol and benzene for IIb, *p*-aminophenol being insoluble in benzene alone. The objectives were obtained quantitatively without the formation of by-products. Accordingly, it was obviously shown

1) Part II: J. Shibasaki, T. Koizumi, T. Tanaka, and M. Nakatomi, *Chem. Pharm. Bull.* (Tokyo), **16**, 1726 (1968).
2) Location: *4-23 Bunkyo-cho, Nagasaki.*
3) Hereafter, for the compounds of present paper which were also appeared in the previous paper,[1] the same abbreviation will be used.
4) *a*) L. Knorr, *Ber.*, **16**, 2593 (1883); *b*) S. Coffey, J.K. Thomson, and F.J. Wilson, *J. Chem. Soc.*, **1936**, 856; *c*) L. Limpach, *Ber.*, **64**, 970 (1931); *d*) C.R. Hauser and G.A. Reynold, *J. Am. Chem. Soc.*, **70**, 2402 (1948); *e*) J.K. Thomson and F.J. Wilson, *J. Chem. Soc.*, **1935**, 111; *f*) F. Sorm and L. Novotny, *Chem. Listry*, **49**, 901 (1955) [*C.A.*, **49**, 13244*g* (1955)]; *g*) K.N.S. Sastry and P. Bagachi, *Science and Culture*, **18**, 543 (1953) [*C.A.*, **48**, 6443*h* (1954)]; *h*) I.G. Farbenind. A.G., Ger. Patent 571319 (1931) [*C.A.*, **27**, 2692[8] (1933)].
5) *a*) F. Chick and N. Wilsmore, *J. Chem. Soc.*, **93**, 946 (1908); *b*) J.W. Williams and J.A. Krynitsky, "Organic Syntheses," Vol. 21, ed. by A.H. Blatt, John Wiley and Sons, Inc., New York, N.Y., 1941, p. 4; *c*) S. Arikawa, A. Kashioka, and T. Kawamata, *Yuki Gosei Kagaku Kyokai Shi*, **16**, 29 (1958); *d*) A.B. Boese, Jr., U.S. Patent 2152786 [*C.A.*, **33**, 5003[2] (1939)].
6) During the preparation of the present paper, details of the reaction of aniline and diketene were reported by T. Kato and Y. Kubota (*Yakugaku Zasshi*, **87**, 1212 (1967)).

that for the purpose of preparing II, the method of diketene is far preferable to that of acetoacetate.

In all experiments mentioned above, authors detected the products by means of thin-layer chromatography (TLC), which enabled them to distinguish the products in a simple manner. Among II, IV, and corresponding amines examined, TLC afforded the highest *Rf* value with IV, the lowest value with II, and intermediate value with amines without exception. *Rf* values and color reactions in TLC are shown in Table I.

Table I. Comparison of *Rf* Values of Acetoacetanilides (II), Corresponding Ethyl β-Anilinocrotonates (IV), and Their Starting Anilines in Thin-Layer Chromatography[a]

R	Solvent system: benzene–ethyl acetate	R-C6H4-NHCCH2CCH3 (II)	R-C6H4-NHC(CH3)=CHCOC2H5 (IV)	R-C6H4-NH2
H	9 : 1	0.18	0.89	0.69
p-EtO	9 : 1	0.11	0.82	0.36
p-HO	6 : 4	0.23	0.88	0.46
o-HO	6 : 4	0.38	0.83	0.62
m-HO	6 : 4	0.25	0.85	0.53

a) All compounds in this table are detected by spraying 5% aqueous silver nitrate to give either brown, gray, or black spot.

Carbonyl Absorption of Infrared (IR) Spectra of II and IV[7]

The C=O absorption bands of IR spectra were examined with II and IV, which are, as mentioned above, closely related derivatives formed by the reaction of acetoacetate and aromatic amines.

As shown in Table II,[8] II gave two C=O absorption bands at *ca.* 1710 and *ca.* 1650 cm^{-1}, which were unquestionably ascribed to ketone and amide, respectively. Absorption bands of IV in carbonyl region were observed at 1620—1650 cm^{-1} which were considered to be due to -N-C=C-C- structure, similar shift being reported on the absorption of γ-pyridone or 4-quinolone,[9] to which 1638 cm^{-1} was assigned.

Furthermore, in order to make sure of the assignment of these C=O absorption bands of IV to ester group, NMR spectra were also investigated, results of which are given in Table II: methyl protons of ethyl ester group ((a) in Table II), triplet (3H) around 1.3 ppm; methylene protons of ethyl ester group ((b)), quartet (2H) around 4.1 ppm; a proton attached to unsaturated carbon ((c)), singlet (1H) around 4.7 ppm; and protons of methyl group attached to another unsaturated carbon ((d)), singlet (3H) around 1.9 ppm. From these results, ethyl crotonate structure of these compounds were confirmed, and consequently the IR absorption spectra of IV at 1620—1650 cm^{-1} was proved to be C=O absorption band of ester.

7) For the analysis of IR and nuclear magnetic resonance (NMR) spectra, many helpful suggestions were given by Dr. T. Kitagawa, Faculty of Pharmaceutical Sciences, Nagasaki University, to whom the authors are grateful.

8) In order to examine IR spectra with II and IV, acetoacetanilide (IIc), *o*-hydroxyacetoacetanilide (IId), and *m*-hydroxyacetoacetanilide (IIe) were obtained by diketene method and ethyl β-anilinocrotonate (IVc), ethyl β-(*o*-hydroxyanilino)crotonate (IVd), and ethyl β-(*m*-hydroxyanilino)crotonate (IVe) were prepared by acetoacetate method.

9) S.F. Mason, *J. Chem. Soc.*, **1957**, 4874.

Table II. Infrared and Nuclear Magnetic Resonance Spectral Data of Acetoacetanilides (II), Ethyl β-Anilinocrotonates (IV), and β-Hydroxybutylanilides (I)

R	II: IR[a] $\nu_{C=O}$ (cm^{-1}) Amide	II: IR[a] $\nu_{C=O}$ (cm^{-1}) Ketone	IV: IR[a] $\nu_{C=O}$ (cm^{-1})	IV: NMR[b] (ppm value) (a) (t, 3H)	(b) (q, 2H)	(c) (s, 1H)	(d) (s, 3H)	I: IR[a] $\nu_{C=O}$ (cm^{-1})
H	1662	1705 1716sh	1617 1652	1.30	4.12	4.66	1.98	1664 1674sh
p-EtO	1655	1708	1650	1.32[c] 1.44[c]	4.04[d] 4.16[d]	4.65	1.90	1650 1668
p-HO	1645 1656sh	1708	1620	1.32	4.15	4.65	1.89	1632 1652
o-HO	1618 1640	1707	1633	1.35	4.16	4.79	1.88	—
m-HO	1655	1711sh 1721	1631	1.33	4.15	4.68	2.02	—

abbreviation: t, triplet; q, quartet; s, singlet; sh, shoulder

a) IR spectra of all compounds in this table were measured in KBr except ethyl β-anilinocrotonate, which was measured in liquid.

b) NMR spectra were determined on solution in $CDCl_3$, using tetramethylsilane as internal reference by Hitachi H-60 spectrometer operated at 60 Mc.

c) Two values are due to (a) and methyl protons in ethoxyl group of benzene ring, respective assignment being unknown and the both methyl protons show an equal coupling constant (J=7.2 cps).

d) Two values are due to (b) and methylene protons in ethoxyl group of benzene ring, respective assignment being unknown and the both methylene protons show an equal coupling constant (J=7.2 cps).

Reduction of II to Corresponding β-Hydroxybutyranilides (I)

Reduction of IIa to Ia with nickel catalyst[10] and of acetoacetanilide (IIc) to β-hydroxybutyranilide (Ic) by potassium borohydride at the presence of lithium chloride in tetrahydrofuran[11] have been reported.

In this work, reduction of IIb and IIc were carried out by sodium borohydride in ethanol to give Ib and Ic quantitatively.

It was obvious that the C=O absorption band of ketone observed for II had disappeared from IR spectra of I as shown in Table II. This fact verified that carbonyl of ketone was selectively reduced.

Experimental[12]

Reaction of Aromatic Amines and Ethyl Acetoacetate (Synthesis of Ethyl β-Anilinocrotonates (IV))

The reaction with aniline and *p*-phenetidide was carried out without solvent and the reaction with *o*-, *m*-, and *p*-aminophenol was performed using methanol as solvent. The following example illustrates the synthesis of IV.

Ethyl β-(*m*-Hydroxyanilino)crotonate (IVd)——To a solution of 2.2 g (0.02 mole) of *m*-aminophenol in 10 ml of methanol, 5.2 g (0.04 mole) of ethyl acetoacetate was added and the mixture allowed to stand overnight at room temperature. Methanol and excess of ethyl acetoacetate were removed below 60° *in vacuo*. The resulting residue was dissolved in ether and the solution was successively washed twice with 5% HCl and twice with H_2O. After drying over sodium sulfate, ether was evaporated and the residue was recrystallized. The yield was almost quantitative.

10) G. Ehrhart and H. Ott, U.S. Patent 2830087 (1958) [*C.A.*, **52**, 14662*g* (1958)].
11) P. Pastour and E. Merechal, *Compt. Rend.*, **243**, 166 (1956) [*C.A.*, **51**, 2598*g* (1957)].
12) Melting and boiling points are all uncorrected.

Solvents for recrystallization, melting points, and the results of elementary analyses of synthesized IV are shown in Table III.

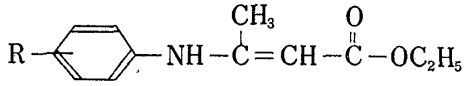
Reaction of Aromatic Amines and Diketene (Synthesis of Acetoacetanilides (II))

IIc was prepared by the reaction of diketene and aniline in the solvent of benzene according to the literature[5b] and IIa was synthesized from diketene and *p*-phenetidine in a similar manner. In the case of reaction with *o*-, *m*-, and *p*-aminophenol, the mixed solvent of MeOH and benzene was employed to dissolve these aminophenols. The following example illustrates the preparation of hydroxyacetoacetanilides.

***o*-Hydroxyacetoacetanilide (IId)**——To a solution of 2.2 g (0.02 mole) of *o*-aminophenol in 20 ml of MeOH, 30 ml of benzene was added and further, 0.7 g (0.02 mole) of diketene was added dropwise with stirring under gentle refluxing. After refluxing for additional 2 hr, the solvent was removed under reduced pressure and H_2O was added to the residue. The resulting precipitates were collected, dried, and recrystallized. The yield was almost quantitative.

Solvents for recrystallization, melting points, and results of elementary analyses of II are given in Table IV.

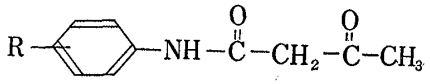
Table III. Characteristics of Ethyl β-Anilinocrotonates (IV)



R	Compd. No.	mp (°C)	Recryst. solvent	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
H	IVc	oil[a]	——	$C_{12}H_{15}O_2N$	70.24	7.32	6.83	69.74	7.09	6.76
p-EtO	IVa	55—58[b]	petr. ether	$C_{14}H_{19}O_3N$	67.47	7.63	5.62	67.08	7.49	5.58
p-HO	IVb	90—91[c]	benzene–petr. ether	$C_{12}H_{15}O_3N$	65.16	6.79	6.33	65.11	6.64	6.37
o-HO	IVd	57—60	benzene–petr. ether	$C_{12}H_{15}O_3N$	65.16	6.79	6.63	65.03	6.69	6.31
m-HO	IVe	106—108	petr. ether	$C_{12}H_{15}O_3N$	65.16	6.79	6.63	65.47	6.38	6.02

a) Purified by distillation, bp 120—121° (3 mmHg) [lit.[4b] bp 137—139° (6 mmHg)].
b) lit.[4b] mp 54—54.5°, lit.[4f] bp 140° (1.5 mmHg)
c) lit.[4f] mp 89—90°

Table IV. Characteristics of Acetoacetanilides (II)



R	Compd. No.	mp (°C)	Recryst. solvent	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
H	IIc	83—84[a]	H_2O	$C_{10}H_{11}O_2N$	67.80	6.21	7.91	67.65	6.17	7.98
p-EtO	IIa	100—102[b]	H_2O	$C_{12}H_{15}O_3N$	65.16	6.79	6.33	65.12	6.70	6.36
p-HO	IIb	91—93	H_2O or benzene	$C_{10}H_{11}O_3N \cdot \frac{1}{2}H_2O$	59.41	5.94	6.93	59.04	6.04	7.03
o-HO	IId	117—119[c]	H_2O	$C_{10}H_{11}O_3N$	62.18	5.70	7.25	61.94	5.42	7.03
m-HO	IIe	130—133[d]	H_2O	$C_{10}H_{11}O_3N \cdot \frac{1}{2}H_2O$	59.41	5.94	6.93	59.25	5.68	6.53

a) lit.[4a] mp 81°, lit.[5b] 84—85°
b) lit.[4e] mp 104—105°, lit.[5c] mp 101—102°
c) lit.[4g] mp 128°
d) This substance appeared in lit.[4h] but mp was not available.

Reduction of II to I

N-(β-Hydroxybutyro)-*p*-aminophenol (Ib)——To a solution of 10 g of IIb in 5 ml of EtOH, was added dropwise with stirring a solution of 1.5 g of $NaBH_4$ in 40 ml of EtOH at room temperature. The reaction was slightly exothermic. The mixture was kept for 2 hr at room temperature and EtOH was removed under reduced pressure. The resulting viscous residue was acidified by 10% acetic acid, saturated with ammonium sulfate, and extracted with ethyl acetate. The extract was dried over sodium sulfate and the

solvent was evaporated to leave reddish brown residue. Recrystallization from hot water gave colorless needles, mp 87—90°. Yield before purification was almost quantitative. After drying on phosphorus pentoxide *in vacuo* at 60° for 48 hr, melting point raised to 116—120°. Elementary analyses of the substance of the lower melting point was consistent with Ib with a half mole of H_2O. *Anal.* Calcd. for $C_{10}H_{13}O_3N \cdot \frac{1}{2}H_2O$: C, 58.82; H, 6.86. Found: C, 58.98; H, 7.07. Elementary analyses of the substance of the higher melting point corresponded to anhydrous Ib. *Anal.* Calcd. for $C_{10}H_{13}O_3N$: C, 61.54; H, 6.67; N, 7.18. Found: C, 61.16; H, 6.58; N, 7.27.

β-Hydroxybutyranilide (Ic)——Ic was obtained by the reduction of IIc with the same procedure described above. The crude product was obtained quantitatively. Recrystallization from benzene gave colorless needles, mp 109° (lit.[11] mp 119°; with 1 mole H_2O, mp 112—113°). According to the results of analyses, Ic thus obtained was identical with anhydride. *Anal.* Calcd. for $C_{10}H_{13}O_2N$: C, 67.04; H, 7.26; N, 7.82. Found: C, 66.76; H, 7.04; N, 7.82.

Ia was supplied by Takeda Chemical Industries, Ltd., mp 157—158° (lit.[10] mp 160°).

Thin-Layer Chromatography

Silica gel B-5 of Wako Pure Chemical Industries, Ltd. was spread about 250 μ thick and dried at 110° for 1.5 hr. Solvent systems and spraying reagent used are shown in Table I.

Acknowledgement The authors are grateful to Mrs. H. Mazume for elementary analyses, Miss K. Ota for NMR and Mr. H. Ishikawa for IR measurements. This work was partly supported by the Grant-in-Aid for Scientific Research from the Ministry of Education, to which the authors are grateful. The authors wish to express their deep gratitude to Takeda Chemical Industries, Ltd. for the kind supply of bucetin.

[Chem. Pharm. Bull. 16(11)2273—2277(1968)]

UDC 615.033/.034 : 615.254

Drug Absorption, Metabolism, and Excretion. IV.[1] Pharmacokinetic Studies on Renal Transport. (1). Simultaneous Chemical Reaction and Diffusion (SCRD) Model for Uphill Transport

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(Received January 11, 1968)

In 1964 an excretion study of sulfonamides from the human kidney was carried out by one of the authors of the present report, and it was observed that five among seventeen slufonamides were secreted to renal tubules besides the glomerular filtration.[3] Since the drug concentration in the renal tubular fluid is much greater than that in plasma, the renal tubular secretion of the drug must have been performed against the concentration gradient. This is uphill transport. Despopoulos[4] suggested that the tubular secretion of sulfonamides was carried out by the same process as *p*-aminohippuric acid (PAH) secretion.

As for PAH, the functional characteristics of the tubular transport process involved have been studied extensively, particularly with respect to the tubular excretion.[5]

According to Beyer,[6] the process of PAH secretion is as follows, Fig. 13 of his review being reproduced in Fig. 1 of this report.

1) Part III: J. Shibasaki, T. Koizumi, T. Tanaka, and M. Nakatomi, *Chem. Pharm. Bull.* (Tokyo), **16**, 2277 (1968).
2) Location: *a*) *4-23 Bunkyo-cho, Nagasaki*; *b*) *Ann Arbor, Michigan, U.S.A.*
3) T. Koizumi, T. Arita, and K. Kakemi, *Chem. Pharm. Bull.* (Tokyo), **12**, 428 (1964).
4) A. Despopoulos, and P.X. Callahan, *Am. J. Physiol.*, **203**, 19 (1962).
5) I. Sperber, *Pharmacol. Rev.*, **11**, 109 (1959); J.V. Taggart, *Am. J. Med.*, **24**, 774 (1958).
6) K.H. Beyer, *Pharmacol. Rev.*, **2**, 227 (1950).