Synthesis of 2′-Acetoxybiphenyl-2-carboxylic Acid and Its Derivatives as Potential Anti-Inflammatory and Analgesic Agents

ALEX GRINGAUZ

Abstract □ 2′-Acetoxybiphenyl-2-carboxylic acid and a series of derivatives were synthesized. The title compound and its unsubstituted amide had both anti-inflammatory and analgesic properties. Its aminoethyl esters exhibited only analgesia. None of the compounds showed any significant antimicrobial activity.

Keyphrases □ 2′-Acetoxybiphenyl-2-carboxylic acid and derivatives—synthesized and evaluated for anti-inflammatory and analgesic properties and antimicrobial activity □ Anti-inflammatory agents—2′-acetoxybiphenyl-2-carboxylic acid and derivatives synthesized and evaluated □ Analgesic agents—2′-acetoxybiphenyl-2-carboxylic acid and derivatives synthesized and evaluated □ Antipyretic agents—2′-acetoxybiphenyl-2-carboxylic acid and derivatives synthesized and evaluated

The search for effective nonsteroidal anti-inflammatory agents with or without analgesic and antipyretic properties is a continuous one. Indomethacin (1, 2), phenylbutazone (3), mefenamic acid (4), and flufenamic acid (5) are relatively recent examples. Each, however, has disadvantages—some serious—in clinical use.

Salicylic acid and its acetyl derivative, aspirin (I), are probably the oldest and most extensively exploited leads for research in this area. One text (6) gives data on 27 aspirin derivatives and analogs that have been marketed over the years.

Homologs and vinylogs also have been synthesized and evaluated recently (7, 8), and numerous salicylic acid derivatives have been prepared and tested for their anti-inflammatory effects (9). In spite of the extensive work over the last 75 years, many innovative approaches to modify the structure are still possible. The latest apparently successful derivative is flufenisal (4′-fluoro-4-hydroxy-3-biphenylcarboxylic acid acetate) (II) (10, 11).

When the *ortho* relationship of the hydroxy or acetoxy functions to the carboxyl group of aspirin-like compounds is altered, products devoid of analgesic and anti-inflammatory properties invariably result. Therefore, it was of interest to determine the effect of "spreading out" the carboxyl and acetoxy groups as they are in aspirin by placing them on different rings of the biphenyl system. Examination of molecular models indicates that the distance between the two groups in I and the high energy conformation 2′-acetoxybiphenyl-2-carboxylic acid (III) are not significantly different.

The normally favored conformation of *ortho*-substituted biphenyls would place the aromatic rings in planes approximately perpendicular to each other. In aspirin the functional groups project from a single plane. Since the title compound exhibits both pharmacological properites of aspirin, it is interesting to speculate on the requirements of the anti-inflammatory–analgesic receptors. The possibility of an interaction with a relatively planar conformation of III should not be ruled out.

RESULTS AND DISCUSSION

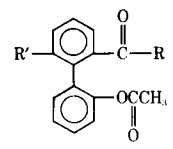
Chemistry—Compound III was prepared by the reactions in Scheme I. 9-Fluorenone was oxidized by a modified Baeyer–Villig-

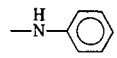
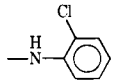
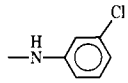
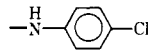
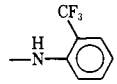
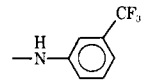
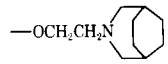
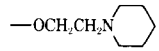
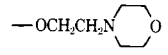
Table I—Pharmacological Properties of 2′-Acetoxybiphenyl-2-carboxylic Acid and Derivatives

Compound	Carrageenan Edema Single Dose, 0.324 mmole/kg po[a], % Inhibition	Adjuvant Arthritis 6-Day Test, 0.324 mmole/kg po[a], % Inhibition	Acetylcholine Writhing, % Inhibition		Antimicrobial Activity
			Oral[b]	Subcutaneous[c]	MIC, g/ml[d]
III	52[e]	45	40	53	125
IV	50 (at 0.648 mmole/kg)	20	Inactive	Inactive	62.5[f]
V	53	62	53	27	—
VI	37 (at 1.11 mmoles/kg)	—	Inactive	Inactive	125
VII	Inactive	—	Inactive	Inactive	125
VIII	49 (at 1.11 mmoles/kg)	—	Inactive	Inactive	125
IX	Inactive	—	Inactive	Inactive	125
X	Inactive	—	Inactive	Inactive	—
XI	34 (at 1.11 mmoles/kg)	—	Inactive	Inactive	—
XII	Toxic[g]	—	—	—	—
XIII	Inactive	Inactive	—	—	—
XIV	Inactive	Inactive	53[h]	87	500
XV	36	Inactive	33	67	—
XVI	13	Inactive	53	87	—
Aspirin	40[i]	51[h]	71[j]	39[j]	—
Phenylbutazone	55	86	100[j]	—	—
Indomethacin	34[k]	80[l]	—	—	—

[a] 0.324 mmole = 100 mg of phenylbutazone. [b] Oral dose = 200 mg/kg. [c] 100 mg/kg. [d] Minimum inhibitory concentration on synthetic Staphylococcus medium against the following organisms: *Saureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* (39645), *Escherichia coli* (198), and *Proteus vulgaris* (9920). [e] Asbestos granuloma 7-day test showed 27% inhibition. [f] Nalidixic acid is active at 5–10 μg/ml. [g] Seven of eight animals died at 100 mg/kg. [h] At 300 mg/kg (1.67 mmoles). [i] At 150 mg/kg (0.833 mmole). [j] ED_{50}. [k] At 3 mg/kg. [l] At 2 mg/kg.

Table II—Physical Properties of 2′-Acetoxybiphenyl-2-carboxylic Acid and Derivatives



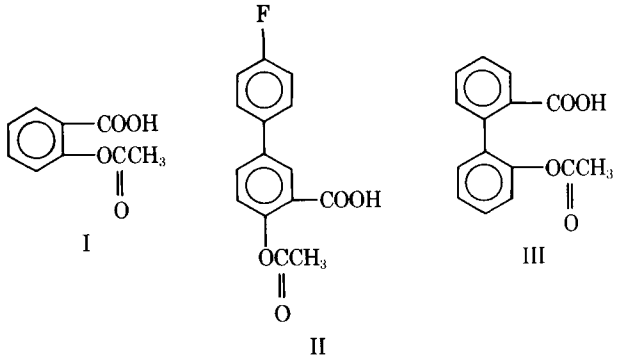
Compound	R	R′	Formula	Melting Point	Analysis, % Calc.	Found
III	—OH	H	$C_{15}H_{12}O_4$	147.5–148.5°	C 70.37 H 4.72	70.27 4.74
V	—NH_2	H	$C_{15}H_{13}NO_3$	162.5–163°	C 70.58 H 5.13 N 5.49	70.42 5.17 5.44
VI		H	$C_{21}H_{17}NO_3$	108–108.5°	C 76.12 H 5.17 N 4.23	75.71 5.21 4.05
VII		H	$C_{21}H_{16}ClNO_3$	139–139.5°	C 68.95 H 4.41 Cl 9.69 N 3.83	68.96 4.33 9.89 3.80
VIII		H	$C_{21}H_{16}ClNO_3$	104–104.5°	C 68.95 H 4.41 Cl 9.69 N 3.83	69.17 4.31 9.84 3.76
IX		H	$C_{21}H_{16}ClNO_3$	148–149°	C 68.95 H 4.41 Cl 9.69 N 3.83	69.02 4.40 9.66 3.74
X		H	$C_{22}H_{16}F_3NO_3$	130–131°	C 66.17 H 4.08 F 14.27 N 3.51	66.31 4.02 14.40 3.35
XI		H	$C_{22}H_{16}F_3NO_3$	109–110.5°	C 66.17 H 4.08 F 14.27 N 3.51	66.30 4.10 14.21 3.51
XII	$-N(C_2H_5)_2$	H	$C_{19}H_{22}NO_3$	93–93.5°[a]	C 73.05 H 7.10 N 4.48	73.26 6.87 4.46
XIII	$-N[CH(CH_3)_2]_2$	H	$C_{21}H_{25}NO_3$	112–113°[a]	C 74.31 H 7.42 N 4.13	74.70 7.53 4.07
XIV		H	$C_{25}H_{29}NO_4 \cdot HCl$	152.5–154°	C 67.63 H 6.81 Cl 7.99 N 3.15	68.13 6.89 8.28 3.17
XV		H	$C_{22}H_{25}NO_4 \cdot HCl$	114.5–115°	C 65.42 H 6.49 N 3.47	64.76[b] 6.70 3.25
XVI		H	$C_{21}H_{23}NO_5 \cdot HCl$[c]	128.5–130°	C 62.14 H 5.96 Cl 8.73 N 3.45	62.35 5.94 8.83 3.40
XVIII	—OH	COOH	$C_{16}H_{12}O_6$	215–216°	C 62.50 H 4.20	62.86[d] 4.09

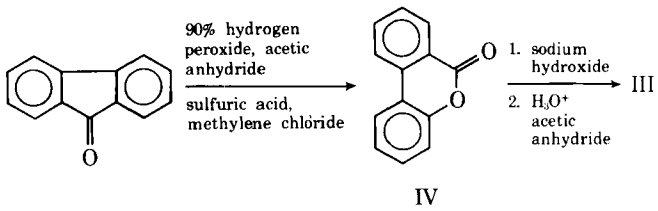
[a] Recrystallized from absolute ethanol four times. [b] TLC detected no impurities; NMR was consistent. [c] Extremely hygroscopic. [d] NMR spectrum was consistent with assigned structure, showing seven aromatic H, two acidic H, and three methyl H; mass spectrum showed a parent peak at *m/e* 300 (mol. wt. 300.3) and *m/e* 256 ($-CO_2$).

er reaction to 2′-hydroxybiphenyl-2-carboxylic acid lactone (3,4-benzocoumarin) (IV) in good yield. Hydrolysis of IV with 2 moles of ethanolic sodium hydroxide, followed by acetylation and careful acidification of the sodium salt of III, afforded the product in satisfactory yield. The acid chloride III*a*, readily prepared with thionyl chloride, was utilized to synthesize the anilides VI–XI as well as the amides V, XII, and XIII. The aminoester hydrochlorides XV and XVI were similarly derived from III*a*.

Compound XIV was prepared by direct reaction of III with 3-(2-chloroethyl)-3-azabicyclo[3.3.2]nonane. The treatment of diphenic acid with hot sulfuric acid afforded 9-fluorenone-4-carboxylic acid (12), which, when reacted as per Scheme I, afforded XVIII. Attempts to oxidize 2,7-dibromo-9-fluorenone to the corresponding lactone failed due to very poor solubility, even in 98% H_2SO_4. Hawthorne and Mihelic (13) reported the facile conversion of diphenic acid to IV by lengthy refluxing with hydrogen peroxide in aqueous sodium bicarbonate or calcium hydroxide. All attempted modifications of this reaction with 4,4′-dichloro- and 6,6′-dimethyldiphenic acids failed to give the desired lactones.

Biology—The anti-inflammatory activity of the compounds was





Scheme I

assessed by their ability to inhibit rat hindpaw carrageenan edema (14), by the adjuvant arthritis test (15, 16), and by the asbestos granuloma test (17). Analgesic activity was determined by the acetylcholine writhing method (18). Antimicrobial activity was ascertained by the tube dilution test, determining the minimum inhibitory concentration (MIC) in micrograms per milliliter (Table I).

As can be seen from Table I, III and V showed significant anti-inflammatory activity in at least two test systems. The analgesic potency of III is probably less than one-fourth that of aspirin. However, the compound is highly hypothermic. The latter property might be expected to prevent it from exhibiting clinically useful antirheumatic activity. The diacid XVIII showed no anti-inflammatory properties. This result might be expected since additional acid functions, such as carboxyl, tend to lower biological activity sharply (19). The three aminoethyl esters XIV, XV, and XVI exhibited analgesic activity but insignificant anti-inflammatory properties.

The data suggest that III and some of its derivatives show interesting and potentially useful activity in the anti-inflammatory–analgesic area. Expansion of this series may lead to more useful compounds and help clarify the structure–activity relationships.

EXPERIMENTAL[1]

2′-Hydroxybiphenyl-2-carboxylic Acid Lactone (IV)—This compound was prepared by an improved Baeyer–Villiger oxidation of 9-fluorenone (20) in 80–85% yield, mp 93–94° (21).

2′-Acetoxybiphenyl-2-carboxylic Acid (III)—To a solution of 80 g (2 moles) of sodium hydroxide in 50% ethanol was added 196 g (1 mole) of IV. The mixture was heated on a water bath until a syrup was obtained. After cooling (ice–salt), 110 g (1.08 moles) of acetic anhydride was added in portions while stirring was continued for 1 hr. A thick paste resulted. Treatment with cold water, rapid filtration (recovering unreacted IV), and acidification (congo red) of filtrate with hydrochloric acid afforded a light-brown semisolid which solidified on trituration. The crude product was air dried and crystallized from benzene, yielding 200.6 g (78%) of product.

Two additional recrystallizations gave a melting point of 147.5–148.5°; IR: 3.2–3.75 (continuous series, bonded OH), 5.65 (C=O, acetoxy), and 5.90 (C=O, carboxyl) μm; NMR: δ 1.96 (3H, acetoxy CH_3), 7.0–8.0 (8H, aromatic), and 12.5 (1H, carboxyl).

Anal.—Calc. for $C_{15}H_{12}O_4$: C, 70.37; H, 4.72. Found: C, 70.27; H, 4.74. Neutralization equivalent: Calc. 256.3. Found 252.7.

2′-Acetoxybiphenyl-2-carboxylic Acid Chloride (IIIa)—A solution of III in benzene was refluxed with excess thionyl chloride 2 hr. The solvent was removed *in vacuo*, and the residue was washed two times with benzene, giving a quantitative yield. Recrystallization from cyclohexane gave a melting point of 86–88°.

2′-Acetoxybiphenyl-2-carboxamide (V)—To a well-chilled solution of 12.4 g (0.4 mole) of anhydrous ammonia in 100 ml of ether was added a benzene solution of 27.5 g (0.1 mole) of IIIa over 2 hr while a temperature of 5° was maintained. The formed solid was filtered and washed with water. The residual product was recrystallized three times from 2-propanol, yielding 19.1 g (75%), mp 162.5–163°; IR: 2.96, 3.20 (N—H), 5.87 (C=O, acetoxy), and 6.02 (C=O, amide) μm; NMR: δ 1.98 (3H, acetoxy CH_3), 5.5–6.5 (2H, NH_2), and 7–8 (8H, aromatic).

Anal.—Calc. for $C_{15}H_{13}NO_3$: C, 70.58; H, 5.17; N, 5.49. Found: C, 70.42; H, 5.13; N, 5.44.

Compounds XII and XIII were prepared similarly (Table II).

2′-Acetoxybiphenyl-2-carboxanilide (VI)—A benzene solution of 5.36 g (0.0195 mole) of IIIa was added to 3.63 g (0.039 mole) of aniline, and the mixture was refluxed for 2 hr. Filtration of aniline hydrochloride and removal of the solvent *in vacuo* gave a product which solidified on trituration with ether. Recrystallization four times from 2-propanol afforded 3.6 g (56%), mp 108–108.5°; IR: 3.02 (NH), 5.63 (C=O, acetoxy), 5.77, and 5.92 (C=O, amide) μm; NMR: δ 2.0 (3H, acetoxy CH_3), 6.8–7.9 (13H, aromatic), and 8.44 (1H, NH).

Anal.—Calc. for $C_{21}H_{17}NO_3$: C, 76.12; H, 5.17; N, 4.23. Found: C, 75.71; H, 5.21; N, 4.05.

Compounds VII–XI were analogously prepared in similar yields (Table II).

2-(Morpholino)ethyl-2′-acetoxy-2-biphenylcarboxylate Hydrochloride (XVI)—A benzene solution of 16.75 g (0.061 mole) of IIIa and 8.7 g (0.061 mole) of 2-morpholinoethanol was refluxed 2 hr, cooled, and treated with absolute ether. The solid obtained, 19.3 g (78%), was crystallized [ethyl acetate–2-propanol (9:1)] to a constant melting point of 128.5–130°; IR: 3.02 (NH), 5.68 (C=O, acetoxy), and 5.74 (C=O, amino ester) μm.

Anal.—Calc. for $C_{21}H_{23}NO_5 \cdot HCl$: C, 62.14; H, 5.96; Cl, 8.73; N, 3.45. Found: C, 62.35; H, 5.94; Cl, 8.83; N, 3.40.

Compound XV was prepared similarly. Compound XIV was synthesized by reacting 27.9 g (0.15 mole) of 3-(2-chloroethyl)-3-azabicyclo[3.2.2]nonane with 25.6 g (0.1 mole) of III in 2-propanol under reflux (10 hr). Workup and recrystallization from 2-propanol afforded 23.5 g (53%) of product. Attempts to react IIIa with 2-diisopropylaminoethanol gave an oil, which only partially crystallized after 3 months at −5°.

2′-Hydroxy-2,6-biphenyldicarboxylic Acid 2′,6-Lactone (XVII)—Diphenic acid, 44.4 g (0.1 mole), was dissolved in 200 g of concentrated sulfuric acid and maintained for 2 hr at 120°. The solution of 9-fluorene-4-carboxylic acid thus obtained (12) was then oxidized under modified Baeyer–Villiger conditions, omitting methylene chloride as the cosolvent. The 13.9 g (58%) of yellow product was recrystallized from ethanol–petroleum ether (bp 30–60°) (9:1), mp 264–266° dec.; IR: 3.27 (OH) and 5.95 (C=O) μm; NMR: δ 5.5–6.9 (7H, aromatic) and 11.0 (1H, COOH).

Anal.—Calc. for $C_{14}H_8O_4$: C, 70.00; H, 3.36. Found: C, 69.73; H, 3.47.

2′-Acetoxy-2,6-biphenyldicarboxylic Acid (XVIII)—This compound was obtained in 64% yield by alkaline hydrolysis of XVII, followed by acetylation as described for III.

[1] Melting points were determined on a Thomas-Hoover Uni-Melt and are uncorrected. IR spectra were obtained, using KBr pellets, with a Perkin-Elmer 337; UV spectra were obtained on a Coleman-Hitachi 124. NMR spectra were carried out on a Varian A60 in $CDCl_3$, CF_3COOD, or dimethyl sulfoxide-d_6 using tetramethylsilane as the internal reference. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and by Dr. F. B. Strauss, Oxford, England.

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Isolation and Chemistry of Alkaloids from Plants of the Family Papaveraceae LXVII: *Corydalis cava* (L.) Sch. et K. (*C. tuberosa* DC)

V. PREININGER, R. S. THAKUR *, and F. ŠANTAVÝ ˣ

Abstract □ From *Corydalis cava* (L.) Sch. et K. (*C. tuberosa* DC) (Papaveraceae: genus *Corydalis* Med.), a mixture of alkaloids (0.53%) was isolated. The main alkaloid was (+)-bulbocapnine, and the minor alkaloids were coptisine, (+)-domestine, adlumidiceine, (+)-predicentrine, protopine, (−)-capnoidine, (+)-stylopine, (+)-isoboldine, 8-oxocoptisine, 1,2-methylenedioxy-6a,7-dehydroaporphine-10,11-quinone, and corysamine. In addition, fumaric acid was obtained. Coptisine, adlumidiceine, predicentrine, 8-oxocoptisine, corysamine, 1,2-methylenedioxy-6a,7-dehydroaporphine-10,11-quinone, and isoboldine were found in *C. cava* for the first time, but rhoeadine and papaverrubine alkaloids were not detected. Predicentrine and isoboldine were identified on the basis of the UV, IR, mass, and PMR spectra.

Keyphrases □ *Corydalis cava*—alkaloids isolated, UV, IR, PMR, and mass spectra □ Alkaloids—isolated from *Corydalis cava*, UV, IR, PMR, and mass spectra

During the Middle Ages, the tubers of *Corydalis cava* (L.) Sch. et K. (*C. tuberosa* DC) (Papaveraceae: genus *Corydalis* Med.) (1) were used for headache, neuroses, tremor, pain, and paralysis of the extremities (2). So far, 20 alkaloids have been isolated (3, 4), and Trabert and Schneidewind (5) isolated bulbocapnine as the main alkaloid (97%). The other alkaloids were (+)-stylopine, corydaline, an alkaloid of the formula $C_{18}H_{19}NO_5$, mp 230°, $[\alpha]_D^{20}$ −112.5° (chloroform), and an alkaloid with a melting point of 225–226°. Manske (6) isolated capnoidine, domestine (nantenine), and narcotine, and Blaschke *et al.* (7) detected sinoacutine.

DISCUSSION

As part of a systematic investigation of the alkaloids of the genus *Papaver* and *Corydalis*, *C. cava*, which flowers in abundance in the meadows and woods, was studied using chromatographic methods. In addition to the alkaloids isolated earlier, *i.e.*, (+)-bulbocapnine (53.1%) (3–7), (+)-domestine, protopine, (+)-stylopine, and (−)-capnoidine, the alkaloids coptisine (according to quantity the second main alkaloid), adlumidiceine (8), (+)-predicentrine, (+)-isoboldine, corysamine, 8-oxocoptisine, and 1,2-methylenedioxy-6a,7-dehydroaporphine-10,11-quinone were isolated. The last two substances are new alkaloids which have not been isolated previously.

In the material studied, it was not possible to detect corydine, corytuberine, glaucine, hydrohydrastinine, (+)-canadine, corybulbine, sinoacutine, isocorybulbine, corydaline, dehydrocorydaline, (+)-corypalmine, scoulerine, (+)-isocorypalmine, thalictricavine, corycavamine, corycavidine, corycavine, and narcotine, which had been found previously (3, 4). Neither was it possible to detect the rhoeadine or papaverrubine alkaloids, which are characteristic for the genus *Papaver* (4). An attempt was made to identify some of the earlier isolated alkaloids by comparison of their physical constants. Thus, it was found that the previously isolated (5, 9, 10) alkaloid with a melting point of 230° might be capnoidine (237°), and the previously isolated (9) alkaloid with a melting point of 137° might be domestine (138°).

The structure of the alkaloid called predicentrine (I) (1,9,10-trimethoxy-2-hydroxyaporphine) (11, 12), whose methylation with